U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ENTOMOLOGY—BULLETIN No. 70.
L. O. HOWARD, Entomologist and Chief of Bureau.

REPORT OF THE MEETING

OF

INSPECTORS OF APIARIES,

SAN ANTONIO, TEX., NOVEMBER 12, 1906.

ISSUED JUNE 17, 1907.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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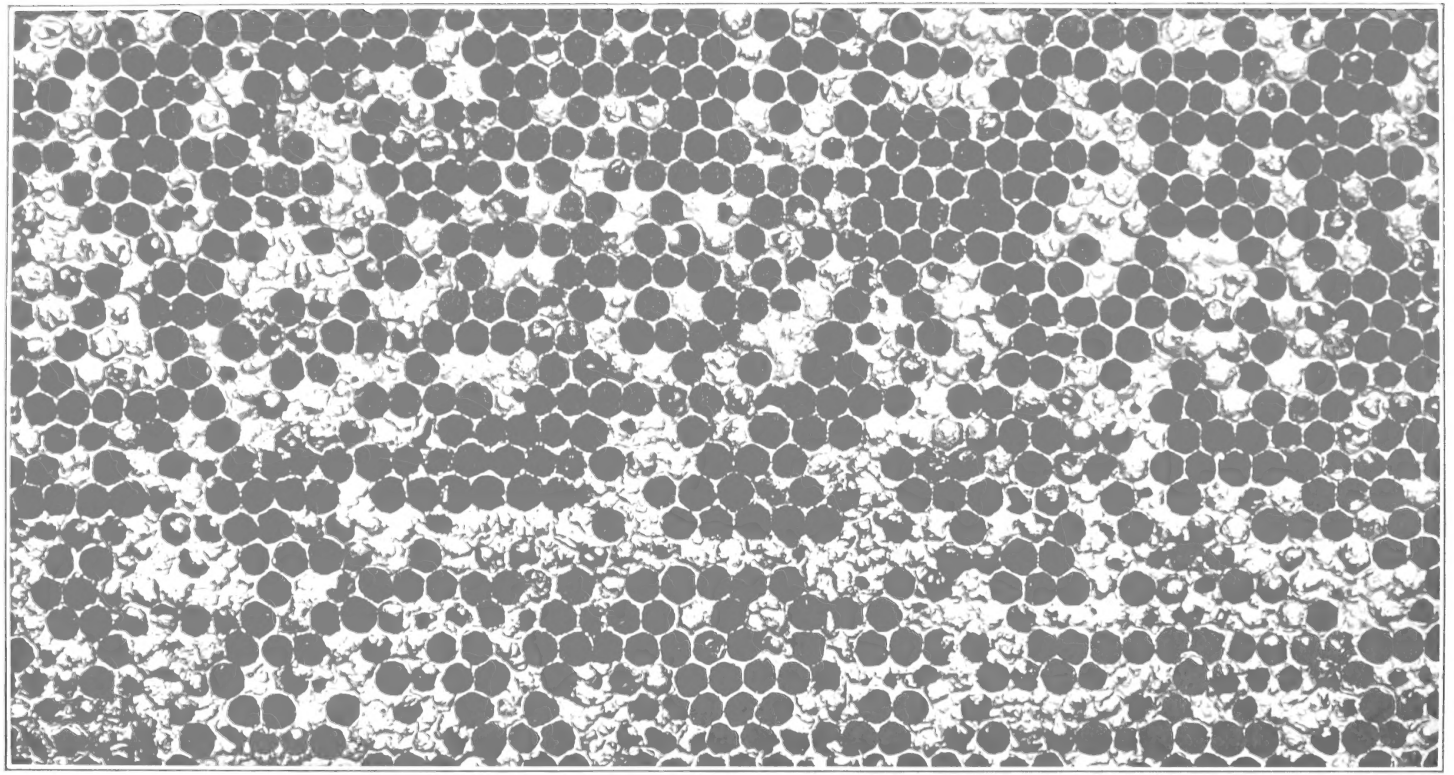

PLATE I.

PORTION OF COMB FROM COLONY INFECTED WITH AMERICAN FOUL BROOD.

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BUREAU OF ENTOMOLOGY.

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C. L. MARLATT, *Entomologist and Acting Chief in absence of Chief.*
R. S. CLIFTON, *Chief Clerk.*

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MABEL COLCORD, *librarian.*

APICULTURAL INVESTIGATIONS.

E. F. PHILLIPS, *in charge.*
G. F. WHITE, *expert in bacteriology.*
J. M. RANKIN, *in charge of apicultural station, Chico, Cal.*
F. G. FOX, *assistant in apiary.*
B. N. GATES, *collaborator, Worcester, Mass.*
JESSIE E. MARKS, *apicultural clerk.*

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Entomology,
Washington, D. C., March 18, 1907.

Sir: I have the honor to transmit the manuscript of the proceedings of a meeting of inspectors of apiaries held in San Antonio, Tex., November 12, 1906. The meeting together and conference of the persons interested in the eradication of the diseases which are such a drawback to apiculture can not fail to bring out many points of importance. In such a meeting the subject is presented in a way which is not possible in articles written for journals devoted to bee keeping or for publication in other forms. There is no organization of inspectors and no funds are available by which these proceedings may be published, and since this meeting was to a large extent the result of the efforts of members of the Bureau of Entomology, and since these men took such an active part in the meeting, it would seem fitting that the proceedings be issued as a publication of the Department of Agriculture. I therefore recommend that this manuscript be published as Bulletin No. 70 of this Bureau.

Respectfully,

L. O. Howard,
Entomologist and Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

The meeting of inspectors of apiaries was held on the Monday following the close of the National Bee Keepers' Association convention, November 12, 1906, at San Antonio, Tex., as a result of a call issued by Mr. N. E. France, inspector of apiaries for Wisconsin, Mr. W. Z. Hutchinson, inspector of apiaries for Michigan, and the writer.

The object of this meeting was to get together for consultation the men interested in the eradication and control of bee diseases. The closer cooperation of these men in their work can result only in good to apiculture and is greatly to be desired. Inspectors are chosen from among the practical bee men of the county or State, and the majority of them are familiar with their work on entering the service. They also accumulate a vast amount of information concerning diseases, most of which never reaches the bee journals or gains publicity in any way. A meeting of these men brings out many points which would otherwise remain unknown.

The meeting at San Antonio was most interesting and valuable, and since much that was said there has never been published it seems desirable to issue the proceedings of the meeting in the form of a bulletin to add to the knowledge of the bee-keeping public on bee diseases.

At the close of the meeting the writer was asked by the inspectors present to prepare a list of questions based on the laws now in force for the control of bee diseases. This list was prepared at once and a copy sent to each of the inspectors whose name and address could be obtained. At the same time the questions were taken up for detailed examination, and various persons were consulted on points of importance which arise. This work is not yet complete, for it has assumed proportions which were entirely unexpected at the beginning. It was originally intended that this discussion of the laws should be inserted as an appendix to the present report, but this would only delay the present publication. This aspect of the subject may therefore be submitted for future publication as soon as it can be prepared. It is very important that the best possible wording be used in a law to control bee diseases. The bee keepers of several States which do not have such laws are at present interested in this subject.

In the preparation of the manuscript for publication it was necessary to rewrite the articles by Doctor White and the present writer because the manner of presentation of these subjects in a meeting is not suited for publication. It was also necessary to edit the discussions, for the stenographic report was inaccurate in numerous places. It is believed, however, that even if the exact words of each person are not recorded, the meaning is the same as was intended to be conveyed. In certain places it has seemed desirable to enlarge somewhat on certain things which were said by the writer. This bulletin therefore can not be called a verbatim report, but it represents nevertheless the proceedings of the meeting.

It is hoped that similar meetings may be held in the future.

E. F. Phillips,
In charge of Apiculture.

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REPORT OF THE MEETING OF INSPECTORS OF APIARIES, SAN ANTONIO, TEX., NOVEMBER 12, 1906.

The meeting was called to order in Market Hall at 9.30 a. m. by Dr. E. F. Phillips, of the Bureau of Entomology, Department of Agriculture, Washington, D. C., who addressed the members as follows:

LADIES AND GENTLEMEN: Last summer it was my pleasure and privilege to visit a considerable number of the men at work on bee-disease inspection throughout the United States. After talking with them and going with them on inspection trips, it became evident that there is a great deal yet to be done in regard to the making of better methods of inspection in work against bee diseases, entirely apart from the scientific aspect of the subject.

On the 3d of last August Mr. N. E. France, inspector of apiaries for Wisconsin, who is attending this meeting, and Mr. W. Z. Hutchinson, the inspector of apiaries for Michigan, and myself met in Milwaukee, Wis., to talk over certain plans for making bee-disease inspection more effective. A circular letter, addressed to the persons now acting as inspectors in the various counties and States of the United States, was drawn up, suggesting that they meet in some place this fall to take up the work of bringing about more uniformity in the methods employed. The meeting of to-day is the result of that circular letter.

There are several things in work against bee diseases that are not at all clear to the bee-disease inspectors and others interested in the subject, and we wish to take some of them up for discussion to-day. We have with us Dr. G. F. White, of the Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C., who has made a most thorough scientific study of the cause of bee diseases, and we shall first ask him to give his demonstrations and the result of his work. After he finishes I shall myself attempt to summarize the investigations of bee diseases which have previously been made by different men. It is perhaps well to take up the scientific aspect of this work first in order to make this clear, and later we shall take up the methods of inspection and treatment and the form of desirable laws controlling bee diseases.

It would perhaps be well for the inspectors of apiaries in the United States to be organized in some way so that there might be greater uniformity in the work and more harmonious cooperation. In Buffalo several years ago such an association was organized. At that time Mr. France, Mr. Hutchinson, and several others met together and organized an association of bee inspectors of the United States and Canada, but they had only an organization meeting and have never met since. Such an organization is desirable, but perhaps not all of those that would care to take part in the association are present.

Doctor White will now give us a demonstration on the brood diseases of bees, a subject on which he has made exhaustive studies.

THE BACTERIOLOGY OF BEE DISEASES.[a]

By G. F. White, Ph. D.,
Of the U. S. Department of Agriculture.

The object of this paper is to discuss briefly the science of bacteriology as it is used in the study of bee diseases and to give a summary of the results of my work on these diseased conditions.

In our discussion of bacteriology, or the science which deals with bacteria, it may be well to consider the subject under the following headings: (1) The nature of bacteria; (2) their distribution; (3) the methods for studying them, and (4) the results of their activity.

THE NATURE OF BACTERIA.

Bacteria are considered by some scientists to be a form of life lower than either animals or plants, but by the majority of authors they are looked upon as plants, and we shall so consider them at this time without going into a detailed discussion of the arguments in favor of such a view. Bacteria, often referred to as germs, microbes, or parasites, are, then, very small plants, so small indeed that they must be magnified 600 diameters or more before they can be seen.

[a] At the meeting of the inspectors of apiaries Doctor White gave a demonstration of the work which he has done on the brood diseases of bees, showing, in illustration of his address, slides and cultures of the various bacteria under consideration. In view of this fact the stenographic report of his address is not clear on all points, since the demonstrations are lacking. It has, therefore, seemed best for Doctor White to write the article here published, giving a popular discussion of this phase of the work which would be intelligible without the demonstration. The substance of his remarks is all included in this paper except the part pertaining to the work of other bacteriologists, which is dwelt on at some length in the article herein published on "The Present Status of Bee Disease Investigation." This method of handling the subject in a published report will make the subject much clearer to those who did not attend the meeting at San Antonio.—E. F. P.

These plants, then, constitute an invisible flora, which we can see only by the use of a microscope of very high magnifying power. The morphology, or the structure, including form and size, is principally of two types, rod-shaped or cylindrical and round or spherical. The size of bacteria varies. Those which are rod-shaped usually measure from 1 to 3–6 microns in length and from one-half to 1 micron in diameter. A micron is the unit of measure for very small objects and is equivalent to $_{25\frac{1}{000}}$ of an inch. For example, if a single bacterium of the rod form measures 2 microns, it would take 12,500 placed end to end to measure 1 inch in length. The spherical bacteria or cocci have about the same diameter as the rod-shaped ones.

Bacteria grow or multiply after a manner called fission; that is, after increasing in size they become constricted in the middle, which constriction finally severs the rod completely, and we then have two bacteria where there was but one before. Under favorable conditions for growth, each bacterium divides by fission every twenty minutes, or, in other words, gives rise to three generations in one hour. Such being their marvelous rate of increase, a little calculation will demonstrate that countless millions may be formed in a short time under favorable conditions, which are proper temperature, moisture, food in correct proportions, and the absence of much light. The temperature most favorable for the growth of a species of bacteria which is able to produce a diseased condition in animals is approximately the temperature of the animal which is affected by such species. Moisture is universally necessary. The food must not be too concentrated. Light inhibits the growth of bacteria. Direct sunlight is bactericidal; that is, it kills bacteria.

Many species produce spores when the conditions are not favorable for the multiplication of bacteria. These are small bodies formed in the bacteria (probably never more than one in a single bacterium) which are somewhat comparable to the grain in wheat and corn. These spores constitute a resting stage and usually also a very resistant stage, for high temperature and strong disinfectant solutions are necessary to kill them. It is these spores which probably make the control of the bee diseases more difficult. When the spores again gain access to a suitable "soil," for example, the body of an animal, they germinate and a new growth takes place as before.

Many species of bacteria have the power to move when they are in a liquid medium, while others do not. This ability to move is due to long, slender processes, which we call flagella, extending from the body of the bacterium.

THE DISTRIBUTION OF BACTERIA.

Bacteria are very widely distributed. Everyone is familiar with the very wide distribution of the higher members of the plant king-

dom, as the trees, shrubs, flowers, and grasses, and we may for convenience refer to these plants as the visible flora. There is also an invisible flora, made up of the plants we can not see except with the aid of the microscope. This flora includes the very minute plants referred to as bacteria, and also the yeasts and some fungi. The distinct species of plants which belong to the invisible flora outnumber by far those which are visible to the naked eye. These microscopic plants are found upon the surface of the animal body and along the digestive tract; they are found in the soil, in the food we eat, and in the water and milk we drink, but are not found within the normal tissues of animals and higher plants.

HOW BACTERIA ARE STUDIED.

The morphology or structure of bacteria is studied with the aid of a microscope of high magnification. Since the number of distinct species of bacteria is so extremely large, and since the shapes assumed by them are so few, it is obvious that many different kinds must look alike under the microscope. This is a point of considerable value in connection with bee-disease work, since in some cases attempts have been made by the use of the microscope alone to determine what species of bacteria was causing certain diseased conditions. With our present knowledge it is not possible to make a positive diagnosis of these diseases with the microscope alone. With the microscope we are able to determine usually only the genus to which any bacterium belongs. If we are trying to identify *Bacillus alvei*, for example, we are able with the microscope alone to say only that it is a Bacillus, since it is seen to be a straight rod. Some other means is necessary to determine the species (*alvei*) to which it belongs. For this purpose we use artificial media or "soils" in which pure cultures of the bacteria are inoculated or planted.

The media in common use are bouillon and sugar-free bouillon, gelatin, agar, and sugar-free bouillon to which has been added small amounts of various sugars known in chemistry as glucose, lactose, saccharose, maltose, and levulose. In addition to these media, use is made of potato, milk, and milk to which litmus has been added, so that the reaction—whether acid, alkaline, or neutral—may be noted. The bouillon is prepared from beef juice to which some peptone and salt are added. Sugar-free bouillon is similar, except that the muscle sugar has been removed. Gelatin is made from pure sheets of gelatin somewhat similar to that used in cooking, to which bouillon is added. The bouillon affords the food for the bacteria or other small plants, while the gelatin keeps the medium solid at ordinary temperatures. Agar-agar (or simply agar) is the dried stem of a certain seaweed which liquefies on heating; to this is added bouillon, as in the

case of gelatin, which solution congeals, as does gelatin, on cooling. The milk used is cow's milk with the butter-fat removed. Before using, all these media are sterilized by heat to kill all bacteria or fungi which might be present.

Having prepared these soils in this way, before the inoculation of them the bacteria must be obtained in pure culture. By pure culture is meant the growth of one species only in a medium. Such a culture is obtained by diluting a small quantity of the material, e. g., decayed larvæ, containing the bacteria with a relatively large amount of liquefied agar, and then pouring it into a shallow sterile glass box (Petri dish). In this way we get only a few bacteria scattered throughout a thin layer of the medium. Each bacterium then begins to grow, and after a few hours it has produced a large number, which, being massed together, we are able to see with the naked eye. This mass of bacteria, having been produced from one individual, constitutes a colony, and such a colony can contain but one species, therefore we speak of it as pure. Pure cultures are then made by inoculations from such a colony. The next step is to identfy this species which we now have isolated from all other species. To do this we inoculate a few or all of the differential media mentioned above. After inoculating and growing the bacteria in these different media or soils at about the body temperature for a day or longer we observe the effect upon the various media produced by the growth of the bacteria and the appearance of the growth in or upon these media. All these phenomena and appearances we speak of as cultural characters. Having obtained in this way the cultural characters of a species of bacteria, we are able to classify it by comparing these cultural characters with the cultural characters of known species.

To illustrate this, let us take for example *Bacillus coli communis*, found normally in the intestine of man and many animals, including the intestine of the adult bee, *Bacillus alvei*, found in European foul brood, and *Bacillus larvæ*, found in American foul brood. *Bacillus coli communis* by its growth in bouillon causes the latter to become heavily clouded; *Bacillus alvei* makes it feebly clouded; while *Bacillus larvæ* does not grow at all in this soil and the bouillon remains clear. In gelatin *Bacillus coli communis* grows very well and does not liquefy the medium by its growth; *Bacillus alvei* grows very slowly and only feebly and liquefies the gelatin; while *Bacillus larvæ* does not grow at all in this medium. When *Bacillus coli communis* is planted on potato it produces a brownish growth; *Bacillus alvei* on this medium produces a lemon-yellow growth, and *Bacillus larvæ* fails to show any growth. When *Bacillus coli communis* is planted in milk there follows a rapid souring of the milk and a firm coagulation of the casein; *Bacillus alvei* produces a soft coagu-

lum which is followed by a slow digestion or liquefaction of the casein: *Bacillus larvæ* does not grow in milk. In litmus milk, *Bacillus coli communis* produces a large amount of acid, which is indicated by the change of the litmus to the red color: *Bacillus alvei* produces no marked change in reaction, and *Bacillus larvæ* does not grow in this medium. In the bouillons to which the sugars, glucose, lactose, saccharose, etc., have been added, there is produced by the growth of *Bacillus coli communis*, gas, and a large amount of acid; *Bacillus alvei* does not produce gas and only a small amount of acid by its growth in the media containing sugars, while *Bacillus larvæ* does not grow when planted in these "soils." (I shall speak later of a medium upon which *Bacillus larvæ* will grow.)

It is by these differences which we observe in the growth upon the various media and the effect produced upon the different media by the growth of the bacteria that we are able to determine one species of bacteria from another.

THE RESULTS OF THE ACTIVITY OF BACTERIA.

In the consideration of this question it is convenient to divide the bacterial flora into two groups—nonpathogenic, or those which do not produce disease, and pathogenic, or those which do produce disease. Some of the nonpathogenic bacteria are economically very important as scavengers. The bodies of dead animals and plants are largely brought to decay by them. The flavors of butter, cheese, and wines are thought to be improved by the growth of bacteria or other micro-organisms—the fungi and the yeasts. Others of these micro-organisms ruin the food, causing the souring of milk, the spoiling of fruit, etc. Many diseases in man and animals are known to be caused by bacteria, as tuberculosis, diphtheria, glanders, and anthrax.

I wish now to speak briefly of how bacteriology has been used in the study of bee diseases, and to summarize the results which have been obtained. For a more detailed account you are referred to a bulletin issued by the Bureau of Entomology of the United States Department of Agriculture—Technical Series, No. 14, "The Bacteria of the Apiary, with Special Reference to Bee Diseases," issued November 6, 1906.

From what has been said one would naturally infer that in every apiary, whether diseased or not, there are on the hives, combs, and bees a large number of bacteria that are perfectly harmless. If one is trying to find in a diseased apiary the species of bacteria which is the probable cause of the trouble, what is the method of procedure? Suppose there were two herds of cattle on adjoining farms and the cattle on one farm were dying while those on the other remained well. If it were suspected that some plant which the cattle were eating was the

cause of death, naturally the plant would be selected which was found on the farm where the animals were sick and which was not found on the farm where the animals remained well. This is exactly the kind of reasoning used when we are looking for the bacteria which are causing the diseases among bees. This necessitates, as you see, the study of all the bacteria which are present in any apiary, whether diseased or not, as well as those in diseased apiaries.

At the time we began the work on bee diseases, in June, 1902, the disorders which were causing the greatest trouble were known to bee keepers as black brood, foul brood, pickle brood, and paralysis. After the study of a large number of samples of brood affected by disease which was being called black brood and the finding of *Bacillus alvei* in all of them, it is very clear that this disease is the same as that investigated by Cheyne in 1885 and called by him "foul brood;" he first described *Bacillus alvei*. "Black brood" was a name given by Dr. William R. Howard, of Fort Worth, Tex., to a disease which he thought existed in New York State, and he described as its cause *Bacillus milii*. After a careful search in New York State for a disease containing *Bacillus milii* we were unable to find it, and there seems to be no ground for the description of a new disease. What has been called black brood by Doctor Howard is obviously the type of foul brood which we now distinguish as European foul brood.

In the decaying larvæ and dried scales found in the cells in the disease which was receiving the name of foul brood there were seen by the use of the microscope a very large number of the spores of bacteria, and in the larvæ in the early stage of the disease there were observed bacteria in the rod form. When these spores were planted upon the media or soils which have been explained earlier in this paper, they would not grow. It became necessary, then, to devise a soil in which the growth could be obtained. After a number of unsuccessful attempts, a medium or soil was made from healthy bee larvæ in which the spores would germinate and the bacteria would grow. By a study of this species, which was found in the dead larvæ of this disease and which was not found in the healthy apiary, it was evident that it was not *Bacillus alvei*, and, since *Bacillus alvei* is not present at all, we know that this disease is not the foul brood which Cheyne had reported in his work in 1885. Since it is not this type of foul brood, what could it be? By carefully reviewing all the work which had been done by others, the conclusion was inevitable that this diseased condition had not been described properly from a bacterial standpoint as a disease separate and distinct from the foul brood of Cheyne, but that the mistake had been made for a long time of calling two different and distinct diseases which affected the brood of bees by one name. This condition was reported to the New York State

department of agriculture in a report to that department made in January, 1903, and another in January, 1904. In the latter report this condition, for want of definite information, was referred to as "X brood" and the bacillus as *Bacillus X*. At the suggestion of Dr. E. F. Phillips, of the Bureau of Entomology, United States Department of Agriculture, it was thought best for very good reasons to retain the name foul brood in the name of each disease and add a qualifying word to designate the difference between the two diseases. "European" is added to foul brood to designate the disease which Cheyne studied in England (Europe) in 1885, and "American" is added to the foul brood which was first studied in the United States (America). We distinguish, then, European foul brood and American foul brood. Both of these diseases of the brood of bees seem to be found in Europe as well as America. It must therefore be remembered that these names do not put any stigma on either country, Europe or America, but, on the contrary, Europe is thereby given the credit of having first studied the European foul brood and America for having first studied American foul brood.

In a study of the so-called "pickle brood" we are unable to suggest from a bacteriological standpoint any cause for the disease. A study has been made of the bacteria found upon the healthy adult bee and those found in the intestine and also the bacteria found upon and within the adult bees suffering with palsy or paralysis, but so far no suggestion can be made from a bacteriological standpoint as to the cause of this disorder of the adult bee.

To conclude, I shall read, with your permission, the summary of the work reported in the bulletin referred to above.[a]

SUMMARY TO PART I.

The results of the study of the bacteria found normally in the apiary may be briefly summarized as follows:

(1) The temperature of the hive approximates that of warm-blooded animals.

(2) Upon adult bees and upon the comb there occurs quite constantly a species of bacteria which we refer to in this paper as *Bacillus A*, and which, it is believed, is the organism that some workers have confused with *Bacillus alvei* which is universally present in European foul brood.

(3) There occurs very constantly in the pollen and intestine of adult bees a species here referred to as *Bacillus B*.

(4) From the combs *Bacterium cyaneus*, *Saccharomyces roseus*, and a Micrococcus referred to here as *Micrococcus C*, have been isolated and studied.

(5) Honey from a healthy hive is, as a rule, sterile.

(6) The normal larvæ are, as a rule, sterile.

(7) There is an anaërobe found quite constantly in the intestine of the healthy honey bee. It is referred to in this paper as *Bacterium D*.

(8) From the intestine there have been isolated and studied the following microorganisms: *Bacillus cloacæ*, *Bacillus coli communis*, *Bacillus choleræ suis*,

[a] Technical Series, No. 14, Bur. Ent., U. S. Dept. Agric.

Bacillus subgastricus, *Bacterium mycoides*, *Pseudomonas fluorescens liquefaciens*, and two referred to as *Bacillus E*, and *Saccharomyces F*. Others less frequently present have been isolated, but not studied.

(9) In two samples of brood with unknown disease there was found a species of yeast plant here referred to as *Saccharomyces G*.

SUMMARY TO PART II.

Following is a brief summary of the results of the present investigation of bee diseases:

(1) There are a number of diseased conditions which affect the apiary.

(2) The disease which seems to cause the most rapid loss to the apiarist is European foul brood, in which is found *Bacillus alvei*—first isolated, studied, and named by Cheshire and Cheyne in 1885.

(3) The distribution of *Bacillus alvei* in the infected hive is as follows:

(*a*) The greatest number of infecting germs are found in the bodies of dead larvæ.

(*b*) The pollen stored in the cells of the foul-brood combs contains many of these infecting organisms.

(*c*) The honey stored in brood combs infected with this disease has been found to contain a few bacilli of this species.

(*d*) The surface of combs, frames, and hives may be contaminated.

(*e*) The wings, head, legs, thorax, abdomen, and intestinal contents of adult bees were found to be contaminated with *Bacillus alvei*.

(*f*) *Bacillus alvei* may appear in cultures made from the ovary of queens from European foul-brood colonies, but the presence of this species suggests contamination from the body of the queen while the cultures are being made and has no special significance.

(4) The disease which seems to be most widespread in the United States we have called American foul brood, and the organism which has been found constantly present in the disease we have called *Bacillus larvæ*. This disorder was thought by many in this country and other countries as well to be the foul brood described by Cheshire and Cheyne, but such is not the case.

(5) From the nature of American foul brood it is thought that the organism has a similar distribution to that of *Bacillus alvei*.

(6) It appears that European foul brood was erroneously called "New York bee disease" or "black brood" by Dr. William R. Howard in 1900.

(7) There is a diseased condition affecting the brood of bees which is being called by the bee keepers "pickle brood." No conclusion can be drawn from the investigation so far as to the cause of the disease.

(8) *Aspergillus pollinis*, ascribed by Dr. William R. Howard as the cause of pickle brood, has not been found in this investigation and is not believed by the author to have any etiological relation to the so-called "pickle brood."

(9) Palsy or paralysis is a diseased condition of the adult bees. No conclusion can yet be drawn as to its cause.

(10) Formaldehyde gas as ordinarily used in the apiaries is insufficient to insure complete disinfection.

CONCLUSIONS.

In a paragraph the author wishes, if possible, to present the status of the bee diseases in this country. It should be remembered, firstly, that "black brood" can now be dropped from our vocabulary, and probably does not exist; secondly, that the term "foul brood" was being applied to two distinct diseases. One of these diseases we now refer to as European foul brood, because it first received

a scientific study from a European investigator. We refer to the other disease as American foul brood, because it was first studied scientifically in America. There is one more disorder in the brood of bees which has attracted considerable attention—the so-called "pickle brood." There are, then, these three principal diseases: European foul brood, American foul brood, and the so-called "pickle brood."

Doctor PHILLIPS. We surely have all been glad to listen to Doctor White in his most interesting account of his work. It will be well at this time to ask him any questions concerning this work which may have come to mind. Before opening this subject for discussion I wish to say that after this discussion I shall take up in detail the works which Doctor White has criticized. Consequently, if you have no objection, we will hold over until later any discussion of these papers.

Mr. C. P. DADANT. As I understand it, there exist these two bacilli (*Bacillus alvei* and *Bacillus larvæ*) and also *Bacillus mesentericus vulgatus*. Have you samples of all three of the bacilli?

Doctor WHITE. Yes, sir; that (pointing to slides) is *Bacillus larvæ;* that (showing cultures) is the *Bacillus alvei*, and the next, *Bacillus mesentericus*. There are a number of varieties of *Bacillus mesentericus*, and *vulgatus* is one of them.

SYMPTOMS OF BEE DISEASES.

Mr. DADANT. Will you please give us a description of the two diseases—that is, of the conditions arising when *Bacillus larvæ* and *Bacillus alvei* are present in the combs?

Doctor WHITE. I should like to ask Doctor Phillips to answer that question.

Doctor PHILLIPS. I shall simply quote from Doctor White's bulletin. There was issued from the Bureau of Entomology some time ago a small circular, Circular No. 79, entitled "The Brood Diseases of Bees," and in this circular was included a description of the two diseases which Doctor White has been studying. Doctor White was kind enough to quote in his bulletin from Circular No. 79, and I shall read the descriptions.

AMERICAN FOUL BROOD.

American foul brood (often called simply "foul brood") is distributed through all parts of the United States, and from the symptoms published in European journals and texts one is led to believe that it is also the prevalent brood disease in Europe. Although it is found in almost all sections of the United States, there are many localities entirely free from disease of any kind.

The adult bees of an infected colony are usually rather inactive and do little toward cleaning out infected material. When the larvæ are first affected, they turn to a light chocolate color, and in the advanced stages of decay they become darker, resembling roasted coffee in color. Usually the larvæ are attacked at about the time of capping, and most of the cells containing infected larvæ are

capped. As decay proceeds, these cappings become sunken and perforated, and, as the healthy brood emerges, the comb shows the scattered cells containing larvæ which have died of disease, still capped. The most noticeable characteristic of this infection is the fact that when a small stick is inserted in a larva which has died of the disease, and slowly removed, the broken-down tissues adhere to it and will often stretch out for several inches before breaking. When the larva dries, it forms a tightly adhering scale of very dark-brown color, which can best be observed when the comb is held so that a bright light strikes the lower side wall. Decaying larvæ which have died of this disease have a very characteristic odor, which resembles a poor quality of glue. This disease seldom attacks drone or queen larvæ. It appears to be much more virulent in the western part of the United States than in the East.

EUROPEAN FOUL BROOD.

European foul brood (often called "black brood") is not nearly as widespread in the United States as is American foul brood, but in certain parts of the country it has caused enormous losses. It is steadily on the increase and is constantly being reported from new localities. It is therefore desirable that bee keepers be on the watch for it.

Adult bees in infected colonies are not very active, but do succeed in cleaning out some of the dried scales. This disease attacks larvæ earlier than does American foul brood, and a comparatively small percentage of the diseased brood is ever capped. The diseased larvæ which are capped over have sunken and perforated cappings. The larvæ when first attacked show a small yellow spot on the body near the head and move uneasily in the cell. When death occurs, they turn yellow, then brown, and finally almost black. Decaying larvæ which have died of this disease do not usually stretch out in a long thread when a small stick is inserted and slowly removed. Occasionally there is a very slight "ropiness," but this is never very marked. The thoroughly dried larvæ form irregular scales which are not strongly adherent to the lower side wall of the cell. There is very little odor from decaying larvæ which have died from this disease, and when an odor is noticeable it is not the "glue-pot" odor of the American foul brood, but more nearly resembles that of soured dead brood. This disease attacks drone and queen larvæ very soon after the colony is infected. It is as a rule much more infectious than American foul brood and spreads more rapidly. On the other hand, it sometimes happens that the disease will disappear of its own accord, a thing which the author never knew to occur in a genuine case of American foul brood. European foul brood is most destructive during the spring and early summer, often almost disappearing in late summer and autumn.

GEOGRAPHICAL DISTRIBUTION.

Mr. FRANCE (Wisconsin). When I was with Doctor Phillips and Inspector Hutchinson in Michigan studying the difference between American and European foul brood, it occurred to me that it was possible to bring together at this time specimens of diseased brood from different localities. In my own city (Platteville, Wis.) I found samples of diseased comb and had reserved them for this meeting, but unfortunately three of the four samples in my possession contained moth larvæ, and it was impossible to tell anything about the disease. The only one that I still have is now in my grip.

Mr. DADANT. Where were those samples from?

Mr. FRANCE. From Michigan.

Doctor PHILLIPS. Our first accurate knowledge of European foul brood in the United States was the epidemic in New York State, and most bee keepers still look on the disease as still being confined to that State. However, European foul brood is now found in New York, Vermont, Massachusetts, Connecticut, New Jersey, Pennsylvania, Ohio, West Virginia, Michigan, Indiana, and Illinois. Reports have been received at the Bureau of Entomology from all those States. The disease is rapidly going west.

COMPARISON OF DISEASES.

Mr. WILLIAM ATCHLEY (Texas). Which of the two diseases is considered worse, the American or the European foul brood?

Doctor PHILLIPS. That is a point which was simply suggested in the descriptions just read. European foul brood spreads more rapidly than the other, but at the same time it will at times absolutely disappear of its own accord, which is something that the American foul brood seems not to do. We have these two factors over against each other, and I should as soon try to eradicate one disease as the other. As far as loss is concerned there seems to be very little difference.

Mr. J. Q. SMITH. (Illinois). How many specimens have been sent to the Bureau of Entomology from Illinois?

Doctor PHILLIPS. Two, I believe.

Mr. SMITH. I was in correspondence with some of the persons having this disease among their bees, and I advised them to send samples to you.

INFECTION IN HONEY.

Mr. R. A. HOLEKAMP (Missouri). Have the bacteria of both diseases been found in honey from infected hives?

Doctor WHITE. We believe that the infecting agent may be present in honey in each case, since the experience of the bee keeper has been that the infection of a healthy colony has followed the feeding of honey from hives affected by either disease.

INFECTION OF LARVÆ.

Mr. A. H. ANDERSON (Utah). How does the larva become infected?

Doctor PHILLIPS. The manner by which this is brought about is not all entirely certain, but the facts would tend to show that it is through feeding on infected material.

Doctor WHITE. It would appear that internally the contamination is caused by being carried by worker bees. As they move over the combs and clean out the cells they come in contact with contaminated material. On any part of the body one can find *Bacillus alvei;* so I shall leave it to you, as you are more conversant with bees.

Doctor PHILLIPS. The bacteria in a diseased colony are present everywhere. They are found all over the adult bees, on the queens, on the outside of the comb, and every place else. They do not, however, grow in honey; they quickly go from the rod condition to the spore condition and remain in the latter condition indefinitely when in honey. According to the statement just made, it would seem that a bee from an infected hive would always carry disease. The fact is, however, that if the bees have been away from this infection for some time they will not transmit the disease. Give them a new clean hive with no food, so that all the honey is used up from the inside of the body. The infection from the outside does not seem to spread the disease if no brood is reared for a few days.

Mr. SMITH. I believe that the reason why the bee loses the infection is because a certain time elapses before the comb is drawn out and young larvæ are present which are large enough to become infected. But I know this fact: If you shake bees from a diseased colony onto combs that contain healthy larvæ, you might as well leave the larvæ, for disease at once appears. I have tried that.

BACTERIA IN QUEENS.

Mr. DADANT. In either case have the bodies of queens been inspected?

Doctor WHITE. The bodies of queens have been inspected, and while the internal organs contained these organisms, the ovaries seldom do, and where *Bacillus alvei* is found in the ovary, or in our cultures made from the ovary, they occur very seldom, and the probability is that they get there through contamination in making the cultures rather than from being found in the ovary itself. The ovaries are very small and one must work with instruments that are sufficiently large to handle. It is almost impossible to take cultures from the ovary and not get contamination from the outside.

"BLACK BROOD."

Mr. H. H. ROOT (Ohio). I thought I understood Doctor White to say that the disease called black brood has not been found in New York.

Doctor PHILLIPS. What Doctor White said was that there is no such thing as black brood. The name black brood was a blunder.

INFECTION CARRIED TO FLOWERS.

Mr. SMITH. Then if, as you say, the contamination is always present on the adult bees from diseased colonies, why is it not possible to carry it to the blossoms and leave it on the pollen, so that the next bee visiting the same flower would carry germs to its hive?

Doctor PHILLIPS. It is, of course, possible, but highly improbable.

VITALITY OF SPORES.

Mr. France. As to the duration of this bacillus in the spore form, how long can it remain in honey and still have vitality to grow under proper conditions?

Doctor Phillips. I have never determined any limit. It is known that very old honey from an infected hive will transmit disease to a colony.

Mr. France. In my State (Wisconsin) we had an experience bearing on this point where combs contained American foul brood. The bees had died, leaving the combs containing dead dried-up larvæ, and the owner, anxious to start in bees again, put the hives away in the granary, and four years afterwards hived bees on them and American foul brood started anew.

Mr. D. H. Coggshall (N. Y.). When honey was shipped from Cuba several years ago and scattered all over the United States, if it was left where bees from this country could get to it, the disease was certainly scattered broadcast.

PUBLICATIONS ON BEE DISEASES.

Doctor Phillips. I desire at this time to announce the publication of three pamphlets on bee diseases issued by the Bureau of Entomology. Circular No. 79, "The Brood Diseases of Bees," was issued three or four weeks ago. I have just this morning received copies of a paper by Doctor White, Technical Series, No. 14, entitled, "The Bacteria of the Apiary, with Special Reference to Bee Diseases." This was issued on November 6, and was received here this morning. I have also a pamphlet here from the Bureau of Entomology containing all the laws in force relative to bee-disease inspection. This is a reprint from Bulletin No. 61, "The Laws in Force Against Injurious Insects and Foul Brood in the United States." A recent order of the Secretary of Agriculture has put a stop to the free distribution of bulletins, but they can be purchased from the Superintendent of Documents, Government Printing Office, Washington, D. C. The price of Technical Series, No. 14, is 10 cents.

The following paper was then read by Doctor Phillips:

THE PRESENT STATUS OF THE INVESTIGATION OF BEE DISEASES.

By E. F. Phillips, Ph D.,
Of the U. S. Department of Agriculture.

I wish to take up two or three phases of this work about which Doctor White has been speaking, and to add some additional points. In regard to the history of the investigation of bee diseases prior to 1885, I can do no better than to quote the historical résumé contained

in a paper by Prof. F. C. Harrison, entitled "Foul Brood of Bees," published as Bulletin 112 of the Ontario Agricultural College:

In all probability the first definite reference to foul brood is by Aristotle (Historia Animalium, Book IX, ch. 27), who mentions an inertness which seizes the bees and causes a bad smell in the hive. He also suggests that bees are liable to become diseased when the flowers on which they work are attacked by blight. Bee dysentery as well as foul brood causes a bad odor; but in the former disease the spotting and consequent smell are usually outside the hive.

Columella (De Re Rustica, Book IX, ch. 13) mentions a bee pestilence and an annual distemper which seizes the bees in spring. Pliny (Natural History, Book XI, ch. 19, A. D. 79) writes of a disease of bees, but as he uses the same term as Aristotle he has probably copied it from the latter author.

Schirach (Histoire des Abeilles, ch. III, p. 56, La Haye, 1771), in 1769, was the first writer to name the disease "foul brood." He says:

It is dangerous and a most destructive disorder to the bees, a genuine plague when the complaint has reached a certain stage. The cause can be attributed to two sources: (1) The putrid (or tainted) food with which the bees feed the larvæ for lack of having better. (2) By the mistake of the queen bee in misplacing the larvæ in their cells, head upside down. In this position the young bee, unable to get out of its prison, dies and rots away.

Further, Schirach clearly distinguishes between foul brood and chilled brood, and mentions the fact that putrefaction follows the death of the brood from frost, but in this case "it is only an accident and not a disease."

The remedy Schirach recommended was to remove all diseased combs from the infected hives and to keep the bees fasting for two days, after which they are furnished with other cakes of wax and a suitable remedy given, "as a little hot water mixed with honey, nutmeg, and saffron, or a syrup composed of equal parts of sugar and wine seasoned with nutmeg." Thus, as Cowan (Journal of the Royal Agricultural Society, Vol. VI, Part IV, 1895) remarks: "We had given us nearly one hundred and thirty years ago a method of cure almost identical with what is by some claimed as new to-day."

Tessier (L'Encyclopedie Methodique, Abeille, p. 32, 1765) about the same time as Schirach says that when the larvæ die in their cells it causes an infection in the hive which makes the bees sick. It is then necessary to drive away or sometimes move the bees from the hive, and to take care to fumigate the infected hive if it is going to be used again. It is necessary, in order to avoid the same inconvenience, to take out the parts of the comb that may be moulded by reason of the dampness. Duchet (Culture des Abeilles, p. 315, Vevey, 1771), who wrote on bees in 1771, does not mention any disease that can be certified as foul brood, but he describes bee dysentery.

Della Rocca (Traite Complet sur les Abeilles, Vol. III, p. 261, Paris, 1790), vicaire-general of Syra, an island in the Levant, relates with much detail the history of an epidemic of foul brood, which caused great destruction in the island during the years 1777 to 1780. Della Rocca describes very minutely the symptoms, destruction, and mistakes that were made in attempting to combat the disease. He says:

The disease manifests its presence by defects in the combs filled with brood, and which only contain a putrid mass; instead of the bee pupæ there is only rottenness in the cells, which, however, being capped, always preserve a healthy appearance. If these cells are broken open, a blackish liquid flows out, which spreads the infection through the hive. This disease only manifests itself in cells which contain a nearly mature larva or a capped one. The bees themselves remain in good health, and work with the same activity, but their numbers decrease daily. This disease, however, was not so general in a hive

but that a small portion escaped. Some new bees emerged, but in too small numbers to supply the daily losses. Thus a hive attacked by this scourge will perish from scarcity of population. At first it was not noticed that this disease was epidemic, and the hives emptied by death of the bees were filled with fresh swarms, and these contracted the same disease and perished. Yet another mistake was made. The remains of the hives that were lost were taken into the streets of the town to expose them to the sun in order to extract all the wax, and the bees from the neighborhood sucked up the honey, caught the disease, and communicated it to other hives, and all, without exception, perished in a short time. The epidemic having reached the island spread everywhere, and the mortality among the bees was general, either from eating infected honey, or from stopping up the infected combs, or from the bees nourishing their brood on infected honey.

Della Rocca criticises Schirach's statement regarding the misplacement of the larvæ by the queen as a cause of the disease, because "everybody knows that the queen has nothing else to do but deposit eggs. These are then cared for and nourished by the bees; and when the larva is nearly ready to change into the pupa, the bees close the cell, and every position which is given the larva depends on their good pleasure and not on the queen's." Della Rocca himself thinks that "some pestilential blight had without doubt corrupted the quality of the honey and the dust from the anthers," and recommends "burning everything without pity, as there is no other resource when the disease is well established, as the pest is without doubt the most terrible in the natural history of bees."

Neither Wildman (Treatise on the Management of Bees, London, 1796), Keys (Ancient Beemasters Farewell, London, 1796, Woolridge), Needham (Brussels Memoirs, Vol. II, 1780, Rhein), Reaumur (Memoirs pour Servir a l'Histoire Naturelle des Insectes, T. V., p. 1734), and other authors about the same time (latter end of the eighteenth century) mention this disease.

Bevan (The Honey Bee, London, 1827) names the disease "pestilence," and also quotes Schirach's name, "foul brood," and says regarding it that the "pestilence has been attributed to the residence of dead larvæ in the cells, from a careless deposition of ova by the queen. * * * It has also been attributed to cold and bad nursing; that is, feeding with unwholesome food."

Nothing further of note appears in bee literature till the year 1860, when Doctor Leuckhart (Bienen Zeitung, Eichstadt, 1860, p. 232) writes that he was formerly of the opinion that foul brood was caused by the same fungus (*Panhistophyton ovatum*) which is noticed in a disease of the silkworm, but now, after observation and experiment, is quite certain that the disease is caused by neither vegetable nor animal parasite. He also notes that the term "foul brood" is applied to a number of diseases affecting bees.

Molitor Muhlfeld (Bienen Zeitung, Eichstadt, 1868, p. 95) recognizes two forms, one contagious and the other not contagious, and thinks that the only cause of contagious foul brood is a fly (*Ichneumon apium mellificarium*) which lays its eggs on the young larvæ of the bee.

A discovery of note was Preuss's (Bienen Zeitung, 1868, p. 225), in 1868. He contradicts Muhlfeld's statement about the fly, and states that foul-brood cells can be detected by the sunken cap. With a microscope magnifying 600 diameters he found small, dust-like bodies, with a diameter of $\frac{1}{300}$ mm., belonging to the genus *Cryptococcus* (Kutzig), and called the specific form *alvearis*, likened it to the fermentation fungus (*Cryptococcus fermentum*), and thought that the last germ gained access to the young bee and changed to *Cryptococcus alvearis*. He notices that many experts lay the cause of the disease to fermenting food, but the larvæ are easily contaminated by the fermentation fungus, which is always present in the air. He also mentions the enormous rapidity with which

the fungus multiplies, and gives an elaborate calculation of the number that might be found in a cell containing a deceased larva.

As might have been expected, Preuss's statement aroused considerable discussion at the meeting of German bee-keepers a short while after the publication of his paper.

Vogel (Bienen Zeitung, Nos. 21 and 22) expressed doubt as to whether *C. alvearis* was the real cause of foul brood or only a consequence of the disease, but on the whole agreed with Preuss.

Wiegand (Bienen Zeitung, Nos. 21 and 22) agreed with Preuss's theory, and in giving his experience said that the disease was introduced into his apiary through honey brought from a distance.

Pollman (Bienen Zeitung, Nos. 21 and 22) believed that the disease was introduced by feeding honey from Havannam, where, when extracting the honey, both brood and honeycomb were mashed up and the honey pressed out.

Doctor Leuckhart (Bienen Zeitung, Nos. 21 and 22) agreed with those who thought the disease due to a fungus, but discredited the supposition that it was the same as the fermentation fungus mentioned by Preuss, and rather thought it was related to the silkworm fungus and that most of the brood diseases ending in death were called "foul brood," while they were really something else. He believed that the fungus was present in the eggs of the queen when laid.

Geilen (Bienen Zeitung, Nos. 21 and 22) believed that the disease came from the putrefying remains of animal bodies upon which the bees alighted.

Muhlfeld (Bienen Zeitung, 1869, No. 3) again, in 1869, presented his former views and also those of Preuss and gave directions for maintaining the health of bees. He recommended the boiling of the honey and a use of carbolic acid in the strength of 1:100, or permanganate of potash 1:300, as disinfectants.

Lambrecht (Bienen Zeitung, 1870, No. 2) thought that foul brood was caused by fermentation of the bee bread.

Hallier (Bienen Zeitung, 1870, No. 2) considered it no specific disease, but thought it was probably produced by different fungi.

Cornallia (Bienen Zeitung, 1870, No. 5) proved contrary to the above and found a fungus which he thought developed foul brood. He called it *Cryptococcus alvearis* and used carbolic acid, potassium permag, and lime water as disinfectants.

Fisher (Bienen Zeitung, 1871, pp. 105–125) advanced a new foul-brood theory in 1871, which somewhat follows the view of Liebig regarding the silkworm disease and plant diseases. According to this theory, the predisposing cause was insufficient nourishment, especially short stores for winter and spring. Shortage of pollen supply was the next cause. Fisher tried to prove his views by the practical experience of bee keepers and explained that the first result of repeated and continued feeding was an increase in the production of bees; and a consequent disproportion between brood and brood feeders arose, which should be looked upon as another cause of foul brood. The disease, he said, might be lessened or exterminated by applying means to reduce the production of brood, as the removing of the queen and the area which the brood occupied. Foul brood is probably the cause of a quantitative dearth of nourishment and a consequent degeneration of the bees. The appearance of fungus growths was only a secondary matter.

Schonfeld (Bienen Zeitung, 1874, pp. 201 and 261) infected several hives with foul brood, and when it had fully developed he took a comb of the rotten brood to the Physiological Institute at Breslau and had it submitted to a microscopical examination by Doctors Cohn and Eidam (Bienen Zeitung, 1874, pp. 201

and 261). This examination showed that in every dead larva and in each foul brood cell, whether the contents were yet white and fluid or brown, tenacious, and ropy, there were to be found long oval bodies, which Preuss called "micrococci." Close to and among them, Cohn was the first to find, with the most powerful of the five microscopes that were used, a countless number of slender pale rods, joined together, and which he at once identified as bacteria of the genus *Bacillus*. The length of a single rod was about 6 micromillimeters, but many of them were two and three jointed, so that these foul brood bacteria microscopically resembled the anthrax bacteria, though of course they were different physiologically and in the manner in which they acted as ferments.

It is not surprising when we remember the state of bacteriological knowledge in 1870, that Preuss should have mistaken micrococci for the spores of a bacillus.

In 1885 the first investigation which merited close attention was published in the Journal of the Royal Microscopical Society, entitled "The Pathogenic History and History under Cultivation of a New Bacillus (*B. alvei*), the Cause of a Disease of the Hive Bee Hitherto Known as Foul Brood," by Frank R. Cheshire, F. R. M. S., F. L. S., and W. Watson Cheyne, M. B., F. R. C. S. One point is here to be especially noticed, there were two authors of this paper. The paper was divided into Part I, Pathogenic History, by Mr. Cheshire, and Part II, History Under Cultivation, by Mr. Cheyne, and with the latter part Mr. Cheshire had nothing whatever to do. Bee keepers are generally giving Mr. Cheshire the credit for this work, but it is clear that Mr. Cheyne, the man who did the bacteriological work, should be the one to get the credit. The description of the disease, contained in Part I, is as follows:

The nature of foul brood as a germ disease.—If a comb be removed from near the center of a healthy hive during the summer months its cells will normally be filled with eggs, larvæ, and pupæ in every stage of development. The eggs as left by the ovipositor of the queen or mother adhere commonly by the end to the base of the cells they occupy, and favored by the high temperature constantly maintained within the hive, the germinal vesicle at about three days matures into a larva ready for hatching. These eggs I have shown as liable to the disease even before they leave the body of the mother, but most careful microscopic examination is needful to make this apparent (and of which I shall speak presently more particularly). On the contrary, the larvæ, which are constantly fed by the workers, so change in appearance soon after infection that a practiced eye at once detects the presence of the disease. Whilst healthy their bodies are of a beautiful pearly whiteness, lying, at first floating, in the abundant pabulum the nurses are ever ready to supply. As they grow they curl themselves at the bottom of the cells until they become too strait for their occupants, which now advance to the head to be in readiness for the cocoon spinning, which follows upon the close of the eating stage. When the disease strikes the larvæ they move uneasily in their cells, and often then present the dorsal surface to its mouth, * * * so that mere posture is no insufficient evidence of an unhealthy condition. The color changes to yellow, passing on by degrees toward a pale brown, whilst the skin becomes flaccid and opaque; death soon occurs, when the body, now shrunken by evaporation, lies on the lower side of the cell, increasing in depth of tone, until in a few days nothing

more than a nearly black scale remains. Should the larvæ, however, escape contamination until nearly the period of pupahood, they are sealed over in the normal way by a cover made of pollen-grains and wax, * * * and which is pervious to air. The cover furnishes a screen, on which part of the cocoon is soon after spread, but the inhabitant of the cell is marked out for death, and before very long the capping or sealing sinks and becomes concave, and in it punctures of an irregular character appear, * * * and this is a nearly conclusive sign of the diseased condition of the colony. The sense of smell is also appealed to, as a peculiar, very offensive, and extremely characteristic odor now escapes from the diseased combs. The bees, in addition, lose energy, but become unusually active in ventilating their hive by standing at the door, heads toward home, and flapping their wings persistently so that a strong outcurrent, and as a necessary consequence, a correspondingly indraught are set up. Should any attempt be made at removing a dead larva which has assumed a deep brown tint, its body tenaciously adhering to the cell wall will stretch out into long and thin strings like half-dried glue. The microscopist can easily explain this. The thin chitinous aerating sacs and tracheæ do not undergo decomposition at all easily, and these remaining, occasion the peculiarity referred to. * * * The disease is terribly infectious, and once started soon spreads from cell to cell and not unfrequently from stock to stock.

Mr. Cheshire was doubtless quite familiar with the disease of the brood and this description of the symptoms, we may assume, was not made from any one case, but from his entire experience. At that time two diseases of the brood were not recognized. We are justified in concluding that both diseases existed in England at that time, as they do now, and doubtless Mr. Cheshire had seen both without realizing that he was dealing with two distinct maladies. In this description he speaks of the disease as attacking brood at various times, for he says: "Should the larva, however, escape contamination until nearly the period of pupahood," etc. His description before that sentence applies as well to what we now call European foul brood as to American foul brood, while the latter description, especially where he speaks of the odor and ropiness, is undoubtedly drawn from experience with what we now call American foul brood. There is, at any rate, ground for the supposition that Mr. Cheshire was dealing with two diseases.

In the case of Mr. Cheyne, however, the case was entirely different. He was probably not familiar with the brood disease from practical experience. He also probably did all his work from one specimen, for he says: "On August 11, 1884, Mr. Cheshire brought to me a piece of comb containing larvæ affected with foul brood, with which I performed the following experiments." In describing this specimen he says: "These larvæ were dead, of a yellowish color, and almost liquid." This description certainly applies as well to European foul brood. Since the original description of *Bacillus alvei* is so important in this work, it may be well to quote entire Mr. Cheyne's part of this paper. This is practically unavailable to bee keepers, since it is con-

tained in a journal to which few bee keepers can have access. It is as follows:

On August 11th, 1884, Mr. Cheshire brought to me a piece of comb containing larvæ affected with foul brood, with which I performed the following experiments: Selecting cells which were closed, but which Mr. Cheshire thought contained diseased larvæ, I brushed them over with a watery solution of bichloride of mercury (1:1000) to destroy the organisms on the outside. With several forceps, that had been heated and allowed to cool, the covering of the cell was picked off so as to display the diseased larvæ. These larvæ were dead, of a yellowish color, and almost liquid, and on examination afterwards their juices were found to contain numerous moving bacilli. By means of a heated platinum wire, tubes of meat infusion rendered solid by gelatin (10 per cent), or by Japanese isinglass, were inoculated from several of these larvæ and kept at a suitable temperature. Development of bacilli, microscopically similar to those seen in the juices of the larvæ, occurred. The characteristics of this development will be presently described. Further, in the tubes, kept at the body temperature, there was not only a development of bacilli but also of spores.

These bacilli, as seen in the larval juices, measure about $\frac{1}{7000}$ inch in length and $\frac{1}{25000}$ inch in breadth. They are rounded or slightly tapering at their ends and often have a clear space near one end. In the juices of the larvæ during life they apparently do not produce spores, although after death spores abound.

In the cultivation in the peptonized meat infusion, rendered solid by agar-agar, the bacilli vary considerably in size, their average length being $\frac{1}{2500}$ inch, some being as small as $\frac{1}{10000}$ inch and others as large as $\frac{1}{3000}$ inch. When they have attained the latter size, division of the rod seems to begin. They are always somewhat pointed at their ends. Their average breadth is $\frac{1}{30000}$ inch, varying from $\frac{1}{35000}$ to $\frac{1}{25000}$.

The spores are largish oval bodies, averaging in length $\frac{1}{12000}$ inch (varying from $\frac{1}{15000}$ to $\frac{1}{10000}$ inch), and in breadth $\frac{1}{23700}$ inch (varying from $\frac{1}{24000}$ to $\frac{1}{25000}$ inch).

In the agar-agar material the spores are generally arranged side by side in long rows, and in old cultivations only a few bacilli can be seen, some forming spores, some without any indication of spores. That these small bacilli can produce such large spores seems at the first glance at a microscopical specimen almost inconceivable, but I have been able to trace on the one hand the development of the spores in the rods, and on the other the sprouting of the spores into adult bacilli. This can be done in the following very simple manner:

Take a number of glass slides, each having a moderate-sized cell hollowed out in its middle; clean it and pass through a Bunsen flame several times to destroy any bacteria on its surface. With a brush apply a little vaseline around the depression, and then place the slide under a glass shade to keep it from the dust. Clean a number of cover glasses, purify them in the flame, and place them on a pure glass plate beneath another shade. With a fine, pure pipette put a small drop of sterilized cultivating fluid (meat infusion with peptone) on the center of each of these cover glasses; then with a fine platinum wire inoculate each of the drops with the spores, or with nonspore-bearing bacilli; rapidly invert them over the cell, press down the cover glass so as to diffuse the vaseline around its edge, and place the slides in an incubator kept at the temperature of the body. These slides are removed at different intervals of time, and as soon as each is taken out the cover glass is turned over and the drop of fluid rapidly dried. The specimen can then be stained, mounted in Canada balsam, and studied at leisure. This method seems to me to be much more satisfactory than the observation of the organisms swimming about in

the drop of fluid, while the specimens can be kept permanently and compared with one another.

In order to study the growth of the spores, I used a cultivation on the agar-agar cultivating material which had been kept at the temperature of the body for fourteen days, and which consisted almost entirely of spores, though a few bacilli were present. As the result of several experiments, I have got a series of preparations which have been taken at various times (15 minutes, 30 minutes, 40 minutes, 1 hour, 1½ hours, 1 hour 50 minutes, 2 hours, 2 hours 20 minutes, 2 hours 50 minutes, 2 hours 55 minutes, 3 hours 20 minutes, 4 hours 20 minutes, 5 hours, 5 hours 35 minutes, 5 hours 40 minutes, and 7 hours 50 minutes), and the course of events is shown in Plate X.[a] The bacilli stain with various anilin dyes—best, I think, with methyl-violet; but the spores resemble the spores of other bacteria in not taking on the stain. The cover glasses on which the organisms are dried are passed three times through the gas flame and floated on the surface of a fairly strong watery solution of methyl-violet for one or two hours. They are then washed in water, and afterwards laid in weak acetic acid (1 per cent) till no more stain comes out. They are again washed in water, allowed to dry at the ordinary temperature, and mounted in Canada balsam. A spore-bearing cultivation shows the bacilli stained violet and the spores unstained, with the exception of their outline, which is of a faint violet color. In most cases no trace of the rod in which the spore was formed can be seen. The first change which is observed on cultivation is that in many cases the outline of the rod in which the spore was formed becomes faintly visible. This can be seen in 15 minutes, and is, I think, simply due to swelling by the fluid, as it is also evident to some extent in the case of spores soaked in water for the same length of time. In from half an hour to an hour it is evident that the bacilli which were present in the original material are beginning to multiply, and a considerable number of rods are now seen containing spores. It is evident that these spores are newly formed, as extremely few bacilli containing spores were seen in the original material, whereas in the preparations taken from a half hour to an hour a considerable number are present. That some of the rods, instead of growing by fission, at once proceed to form spores is probably to be explained in this way. When the cultivation was removed from the incubator, some bacilli were growing by fission, some were forming spores, and some had passed into a state ready to form spores. The first go on growing by fission, the last complete their spore-formation, which was arrested by removal from the warm temperature. That actively growing rods would not have formed spores so early is evidenced by the facts observed in the second series of observations on the formation of spores. The next thing that is observed is that several of the spores take on the stain, and are as intensely violet as the adult bacilli. The number of the spores which take on the stain in this way goes on increasing as time passes, till in about four hours almost all the spores stain violet. In three hours the first indication of sprouting of these spores becomes evident. The stained part of the spore loses its oval shape, becomes elongated, and is soon seen to burst through the spore-capsule at one part. It then presents the appearance of a short rod, with a pale envelope embracing one end. This rod gradually leaves the spore-capsule and then goes on multiplying as a full-grown bacillus. In specimens taken from four to five hours all stages of growth can be seen, and the remains of the ruptured spore-capsules are evident.

The bacilli appear to grow mainly by fission, but I have seen appearances

[a] Not reproduced here.

which seem to me only explicable on the supposition that they also grow by sending out buds from one end. A bacillus may be seen with a small, somewhat conical stained point attached to one end, though separated by a marked division. This is certainly not the common mode of growth by fission, for there the rod seems to divide into two pretty equal halves, while here we have but a minute piece attached to one end.

The mode of formation of spores may be traced in a similar manner to that described above in the case of the sprouting of the spores. It is, however, as a rule, necessary to leave the organisms to grow for a much longer time than in the former instance. I have not found development of spores as a rule before twenty-three hours, but this depends very much, apparently, on the amount of fluid that was present and the number of bacilli introduced at the time of inoculation. The first thing noticeable is that the rod begins to swell and becomes spindle shaped. The swelling, which generally affects the middle of the rod, may in some cases be most marked toward one end, increases in size, and the center of the swelling gradually ceases to take on the stain. The capsule of the spore is apparently also formed within the rod, and is not merely the outer part of the rod. In three or four hours the rod is seen to have almost or completely disappeared, leaving the spore lying free or within the faint outline of the original bacillus. It seems to me that the view that spore formation occurs when the food is getting exhausted is correct, for the time at which this appearance is found depends greatly on the drop placed on the cover glasses, and I have found in one experiment that in one specimen, after twenty-three hours, most of the rods were forming spores, while in another specimen where the drop was much larger there was no trace of spore formation after twenty-eight hours. I have here described the results of my earlier and rougher attempts to study the formation of spores. I have, however, now improved the method in the following way. As I have just now shown, the period at which spores are first seen seems to depend mainly on the amount of fluid used and the number of bacilli introduced, and as in the above method, both these factors vary in each case, one can not get a regular series of preparations showing the different stages at different times. In studying the sprouting of spores the amount of fluid and the number of spores does not matter, for if sufficient nutriment is present and a proper temperature is maintained the spores must sprout, and probably they always take about the same length of time. The difficulty of obtaining a series of specimens illustrating spore formation is easily obviated in the following manner. Take a pure flask containing a small quantity of sterilized infusion and inoculate it from a cultivation containing only bacilli. Place it in the incubator for two or three hours so that the bacilli may increase somewhat in number and diffuse themselves through the liquid. Thus the cultivating material contains bacilli pretty equally diffused through it, and if after shaking the flask drops of equal size are taken, each will probably contain about the same number of bacilli. The minutest quantity of fluid can easily be obtained by means of a syringe having a fine screw on its piston and a large nut revolving on this screw. The circumference of the nut being equally divided into a number of small segments, the same quantity of fluid can always be expelled from the syringe. By proceeding in this way equal-sized drops containing an equal number of bacilli can be used and a regular series of specimens obtained. I have found that using two-fifths of a minim containing one bacillus and keeping the specimen at 36° C., the earliest appearance of spore formation was evident in forty-one hours.

Leaving these matters, which are of great interest not only in regard to the *Bacillus alvei*, but to all spore-bearing bacteria, and which I have therefore

dwelt on at length, we must pass on to the further consideration of this particular organism. The first point to be determined in investigating its relation to foul brood was whether this was a new bacillus, unknown except in connection with this disease of bees, or whether it was a more or less well-known form. To ascertain this point with regard to micro-organisms the microscope is of little use; recourse must be had to the study of their life history, more especially to their peculiarities of growth on different soils. Of all the materials employed as cultivating media, Koch's gelatinized meat infusion is the most useful for purposes of diagnosis. This is composed of an infusion of meat containing 1 to 3 per cent of pepton, 10 per cent gelatin, made neutral by carbonate of soda, and thoroughly sterilized. This material was first introduced with the view of having a highly nutritive solid and at the same time transparent medium on which to carry on pure cultivations, but it was soon found that owing to the remarkably diverse ways in which different micro-organisms grew in it, it could be used as a means of diagnosis of the kind of organism, a means more certain than any other which we at present possess. For purposes of diagnosis, as well as with the view of carrying on pure cultivations, this material is used in three ways. While the material is still fluid a small portion is poured into a number of pure tubes plugged with cotton wool, sterilized, and allowed to solidify. A fine platinum wire, heated in a flame and allowed to cool, is dipped into the material containing the bacterium in question, and then, after the removal of the cotton-wool plug, is rapidly plunged down through the gelatin to the bottom of the tube and then withdrawn. The plug is reinserted and the tube kept at a temperature suitable for the development of most forms of bacteria, but not high enough to melt the gelatin. If growth takes place at this temperature it occurs either on the surface around the point of entrance of the needle, or along the needle track, or in both places, and the appearance of the growth varies remarkably, according to the different species of micro-organisms studied. The second way is to liquefy and pour out a little of the gelatinized material on microscopic slides or on larger plates of glass which have been sterilized by heat. These plates are placed in glass vessels containing moist blotting paper to prevent drying of the gelatin and to protect them from the dust. After the gelatin has solidified the purified platinum needle charged with the bacteria is drawn rapidly over the surface of the gelatin. Bacteria are sown along the track, grow there, and the whole can be placed under a microscope and the characteristics of the growth studied with a low power. In the third mode a tube of the gelatin mixture is inoculated with a very minute quantity of the bacteria. The tube is then placed in water at the body temperature to melt the gelatin. When the material is melted, it is thoroughly shaken up to diffuse the bacteria through it and, while still liquid, is poured out on sterilized glass plates kept in a moist chamber, as in the former case. Solidification very soon occurs, and the bacteria being caught at various parts of the gelatin grow there in the form of groups or colonies, which can be observed under the low power of the microscope. I shall now describe the characteristics of the *Bacillus alvei* when cultivated in these three modes.

(*a*) *Test-tube cultivations.*—If an infected needle be plunged into a tube of gelatinized meat infusion in the manner described above, growth occurs both on the surface and along the needle track. On the surface the bacilli shoot out in all directions from the point of entrance of the needle, forming a delicate ramifying growth on the top of the gelatin; the characteristics of this growth will be presently described under *b*. Along the track whitish irregular shaped masses appear, which slowly increase in size and run together. In a few days

processes are seen to shoot out from these masses, which may extend through the gelatin for long distances from the track, being thickened at various parts and clubbed at the ends. These processes do not appear to join one another at their ends. A very beautiful and characteristic appearance is got where very few bacilli are introduced with the needle and where, therefore, at various parts of the track, more especially at the lower part, individual bacilli or groups of bacilli are planted at a considerable distance from each other. In a few days minute round whitish specks become visible to the naked eye. These increase in size till in about ten days shoots begin to appear. These radiate from the central mass in all directions and become nodular at various parts, as described above. When such a cultivation is old, the white branches disappear, and only little whitish collections of bacilli are seen at various parts. On examining such a tube with the pocket lens, however, numerous watery-looking tracts are seen running through the gelatin from the central mass to the whitish collections. The gelatin at the upper part of the track generally evaporates, to some extent giving rise to the air-bubble appearance so characteristic of the cholear bacillus. These are the appearances seen where the material contains gelatin in the proportion of 10 per cent. Where less gelatin is present, the naked eye appearances, while possessing the same characteristics, are somewhat different. The shoots are much more numerous and appear much more rapidly, giving rise to a haziness around the middle track, which with the pocket lens is seen to consist of numerous delicate branches clubbed at the ends, as in the former case. I think the amount of pepton present also makes a difference in the appearance, though of this point I am not yet absolutely certain. The most characteristic growth is, however, obtained when the material contains 3 per cent pepton as well as 10 per cent gelatin, the shoots being then less numerous and much coarser. And I can easily understand that this would be the case, for the bacilli would have a large supply of nutriment in their immediate vicinity without the necessity of having, so to speak, to spread out through the gelatin in search of food as may be the case where no pepton, or only a small amount, is present. This appearance is quite characteristic of this bacillus and is not seen in the cultivation of any other organism that I know of. The bacilli of anthrax and of mouse septicæmia also spread out from the needle track, but the appearance of their cultivation is quite different. In anthrax delicate threads, not clubbed, shoot out from the track, soon anastomosing with other threads and forming a delicate network throughout the gelatin. In mouse septicæmia the appearance is that of a delicate cloudiness spreading through the gelatin. These foul-brood bacilli, growing in this material, render it liquid after a time, the liquefaction beginning at the surface and only spreading slowly downwards, but ultimately the whole tube becomes liquid. After two or three weeks' growth the appearance presented by the tube is that of a layer of liquid at the upper part, and the growth along the needle track with the other appearances described at the lower part. The liquid portion is clear, except at the bottom of the liquid, where there is a loose white flocculent deposit of bacilli, and on the surface there may be a very thin scum. The liquid becomes yellowish in colour after a time and gives off an odor of stale but not ammoniacal urine, or what may be better described as a shrimpy smell. This yellowish colour and the peculiar odour have been found by Mr. Cheshire to be distinctive of the diseased larvæ.

(*b*) If gelatin be poured out on a plate, allowed to solidify, and then stroked with an infected needle, we learn the explanation of the appearances seen in the test-tube cultivations. The bacilli at first grow along the needle track, but very soon they are seen to be collecting at parts forming pointed processes. From the processes the bacilli grow out into the gelatin, often a single series of rods, in Indian file, or two or three rods side by side. These processes are not quite

straight, but tend to curve, and at a little distance from the track they grow round so as to form a circle. From this circle, which may be formed of single bacilli, the process continues forming a fresh circle farther on. The bacilli in the circle increase in number till ultimately it becomes completely filled up, and we have a nodule consisting of bacilli in the course of the shoot. These shoots may also join one another, forming a curved anastomosis, and the gelatin in the immediate vicinity of the bacilli becoming liquid, a series of channels are formed in the gelatin containing fluid in which the bacilli swim backwards and forwards. Later on parts of these channels become apparently deserted by the bacilli, so that the circles look to the naked eye as if they were detached from the main track, but with a low power of the microscope the empty channels can be traced.

It is impossible to give a proper idea of the appearance of the growth. The forms assumed are the most beautiful shapes I have ever seen, but they are very numerous, always, however, retaining the tendency to form curves and circles; thus we have the explanation of the appearances previously described in the test-tube cultivations.

(*c*) The appearances of the colonies on plates on which the mixture of bacilli and gelatinized infusion has been poured out is also very characteristic. The earliest appearance of colonies is a small oval or round group of bacilli. This group is not homogeneous in appearance under a low power of the microscope, but lines indicating the bacilli are seen in it. It very soon becomes pear-shaped, and from the sharp end of the pear processes begin to pass out into the gelatin as before described.

These bacilli do not grow below 16° C. The best growth in gelatin is obtained at a temperature of about 20° C. They grow most rapidly in cultivating materials kept at the body temperature. Very few spores are formed at the lower temperatures, but they appear rapidly and in large numbers at the body temperature. I have several times observed bacilli containing spores swimming about freely. The reaction of the medium is not of any great importance, but a neutral medium is apparently the best. The bacilli swim freely in fluids with a slow oscillating movement.

They grow rapidly at the body temperature in meat infusion with pepton and rendered solid by agar-agar, but the appearance of their growth is not nearly so characteristic as in gelatin. This, indeed, is the case with most bacteria, so that agar-agar preparations, though very useful for carrying on pure cultivations at the temperature of the body, are of little value for diagnostic purposes. They grow most rapidly on the surface of the agar-agar, forming a whitish layer, but the shoots described above in the case of gelatin do not occur, or only very imperfectly, in agar-agar. Here the bacilli arrange themselves apparently side by side, and, producing spores in this position, we have as a result, after a few days' cultivation, long rows of spores lying side by side with here and there an adult bacillus.

On potatoes they grow slowly, forming a dryish yellow layer on the surface. They grow very slowly indeed at the lower temperature. In order to get good growth it is necessary to keep the potato at the body temperature.

In milk they grow well at the body temperature, and in a few days cease coagulation of the milk, which also assumes a yellowish colour and gives off the odour previously described. The coagulum is not firm, like that caused by the *Bacterium lactis*, but is like a tremulous jelly, and may remain for a considerable time without the separation of any fluid, but ultimately it becomes liquid, and after some months assumes the appearance of a dirty, brownish-yellow, glairy fluid. It is very slightly, if indeed at all, acid.

They grow extremely slowly in coagulated blood serum, though kept at the body temperature, and there form very long filaments with comparatively few spores.

In meat infusion kept at the temperature of the body they grow readily, causing muddiness, and after a few days a slight but not tenacious scum. The same peculiar odor is also developed here, more especially if the infusion contains a considerable amount of peptone. I do not think that there is any change in the reaction of the fluid; I generally make the infusions faintly alkaline, and after the growth of this organism in it it is faintly alkaline.

These characteristics show that this is a new bacillus, and one which, so far as my knowledge and experience goes, is only found in foul brood. The constant presence in large numbers of a characteristic organism in a disease and its absence elsewhere must, according to our accumulating experience, afford a strong presumption that the organism is the cause of the disease. In the case of foul brood this matter has been completely proved by the following experiments, the details of which will be found in Mr. Cheshire's part of this paper. With a cultivation in milk he sprayed a comb containing a healthy brood, allowing the spray to act only on a particular part of the comb. This part and no other became affected with foul brood. He has also succeeded in infecting adult bees by feeding them with material containing these cultivated bacilli.

I have also had the opportunity of watching the effect of feeding flies with material containing spores and bacilli. I was one day testing some milk in which these bacilli were growing; a large blue-bottle fly settled on it and commenced to eat. I at once put a large glass funnel over the insect, leaving plenty of air. When I came to the laboratory twenty-four hours later, the fly was in the sitting posture on the table and was dead. Its juices were full of these bacilli, as shown by microscopical examination and by cultivation.

Other animals which I have tested are more or less refractory to this bacillus. I have kept cockroaches for days in a box in which was milk containing these bacilli mixed up with sugar. I have also kept them in a box containing a piece of paper which had been thoroughly smeared with the spores. None of them died, but I can not be certain that in either case they ate any of the material, for I never saw them even near it.

I inoculated two mice and one rabbit with a spore-bearing cultivation without effect.

I injected half a syringeful of a spore-bearing cultivation into the dorsal subcutaneous tissue of each of two mice. One of these died in twenty-three hours, the other seemed unaffected, but in the second case I doubt whether the full quantity was introduced. In the case of the mouse which died, the seat of injection and the neighbouring cellular tissue was found to be very œdematous, but no microscopic changes were apparent in the internal organs. Numerous bacilli were found in the œdematous fluid, as also a number of spores which had not been sprouted, and there were also a few bacilli in the blood taken from the heart. This was proved, of course, by cultivation as well as by microscopical examination. On examining sections of the various organs no morbid changes were found, and only very few bacilli were seen in the blood vessels.

At the same time that I injected the mice I injected a syringeful of the same cultivation subcutaneously into a guinea pig. This animal died six days later with extensive necrosis of the muscular tissue and skin and cheesy-looking patches distributed through it. There was no true pus. On making sections of the necrosed tissue numerous bacilli, apparently *Bacillus alvei*, were seen,

but there were other bacteria and also micrococci, as of course would be the case on account of the death of the skin. No micro-organisms were seen in the internal organs. It thus remains questionable whether the necrosis was due to the *Bacillus alvei* or not, more especially as I have since injected three guinea pigs subcutaneously with spore-bearing cultivations, but without effect. I must reserve the action of these bacilli on the higher animals for further investigation, as well as several other points of interest in regard to this organism to which I have not here alluded.

I venture to think that when all the evidence brought forward by Mr. Cheshire and myself is carefully weighed no doubt can be entertained that this bacillus is new to science and is the cause of foul brood. Many questions of course still remain open, requiring further investigation into the life history of the disease.

The next investigator to take up a bacteriological treatment of bee diseases was Prof. J. J. Mackenzie, bacteriologist of the provincial board of health of Ontario. The results of this work were published in the Ontario Agricultural College Report for 1892, pages 267–273. At the request of the Bee Keepers' Union of Canada certain things were taken up which had a very practical bearing on the question of eradicating the prevalent disease.

Professor Mackenzie knew of but one disease, probably, and having in hand the work of Cheshire and Cheyne, assumed that the disease found in Canada is the same as that described by Cheyne. This is a natural mistake after the confusion in the diagnosis by Cheshire. It was not the object of this investigation to demonstrate what organism produces the diseased condition, but, assuming that *Bacillus alvei* causes the trouble, to determine what resistance to heat the organism has.

No adequate description, such as would allow us to make any comparisons with *Bacillus alvei*, is included in Professor Mackenzie's paper. We do know, however, or at least have every reason to believe, that European foul brood was not found in Canada at that time and is not prevalent there now. I have been informed personally by Mr. William McEvoy, the veteran inspector of Ontario, that the disease which we now designate as American foul brood is the prevailing disease in Canada. It seems reasonable to suppose, therefore, that the samples taken to Professor Mackenzie by Mr. Holtermann and others did not contain any *Bacillus alvei*.

Professor Mackenzie does not indicate in his paper that he had any difficulty in getting the organism with which he worked to grow on ordinary media. *Bacillus larvæ*, which is present in American foul brood, does not grow in such media, however, so there is but one conclusion to be reached, and that is that he was dealing with some non-pathogenic form and not with *Bacillus larvæ*. Since the bacillus described by Doctor White as *Bacillus A* is found on combs, both diseased and healthy, and somewhat resembles *Bacillus alvei*, it may be that this is what Professor Mackenzie had.

This paper has probably come to the notice of but few bee keepers in the United States, because the report of the agricultural college is not widely distributed. To make it available for comparison, therefore, it is included here.

THE FOUL BROOD BACILLUS (B. ALVEI); ITS VITALITY AND DEVELOPMENT.

[From Ontario Agricultural College Report for 1892, pp. 267–273.]

Mr. J. J. Mackenzie, B. A., bacteriologist of the provincial board of health of Ontario, read the following paper:

GENTLEMEN: At the request of your secretary, Mr. Holtermann, I undertook for your union some investigations on the subject of foul brood, the results of which I propose giving you in this paper. Although it is almost a year now since I undertook this work under the auspices of the Agricultural and Experimental Union, it is by no means exhausted, and there are many points which require to be further elucidated, which I have not had time as yet to touch on, owing to the fact that investigations on foul brood had to be carried on simultaneously with my regular laboratory work. These points I hope to work at next summer, and reserve the privilege of reporting again to your union on the results of further investigation.

The subject of foul brood is an old one to apiarists and an intensely interesting one to Canadian bee keepers, but in reading over the bee journals one can not help being struck with the great want of unanimity amongst bee men as to the disease, how it should be treated, how it is spread, and on many other points. Some would have us believe that the disease arises de novo whenever insanitary conditions prevail; others claim that there is a specific infection and where the disease arises it must have originated from previously existing disease; some claim that the honey is the only method of transmittal; others that it is not, and so on. On every point there seems to be plenty of arguments pro and con.

I have attempted in my work to take hold of some of these controverted points from a bacteriological standpoint in order to aid in coming to some definite conclusion. Some of these points I should consider settled from the results of previous investigation; but as many bee men do not seem prepared to accept this, my work will have value as confirming what has already been done.

Before an association which includes many practical bee keepers it would be superfluous to enter upon a minute account of the clinical features of the disease. Most of you know them better than I do. I certainly would not be prepared to "spot" foul brood in an apiary, although I certainly think I can under the microscope. The infectious character of the disease has been generally accepted for many years, but not until Cheshire and Watson Cheyne worked it out scientifically was it definitely proved. They isolated a bacillus (*Bacillus alvei*) which they found in the diseased brood and which they cultivated on nutrient media for many generations, finally reinfecting perfectly healthy brood from these pure cultures. This evidence to a bacteriologist is absolutely conclusive that *Bacillus alvei* is the specific cause of foul brood. Consequently, when I began my investigations on some samples of diseased brood which were sent me through Mr. Holtermann, I looked at once for *Bacillus alvei*. Microscopically and by means of bacteriological methods I had no difficulty in isolating a bacillus which corresponds in all points to *Bacillus alvei*. It is a bacillus similar to that of Cheshire's in size, produces spores which are somewhat thicker, giving the bacillus a clubbed appearance. On agar jelly it grows rapidly so as to cover the whole surface. In gelatin its growth is very peculiar, shooting out from the infected point in all directions. On potatoes it produces

a yellow growth. All these characters show conclusively that it is identical with *Bacillus alvei*. There seems no doubt, therefore, that the foul brood which we have in Ontario is the same disease and produced by the same bacillus as in other places.

Many prominent bee keepers, both here and in the States, however, maintain that wherever unsanitary conditions are allowed to prevail, wherever chilled brood is allowed to putrefy, or decapitated drones are left to decay in the hive foul brood may arise de novo. This is not a new theory, either in bee keeping or in medicine, but unfortunately it is a theory which is not supported by the results of investigation. Diphtheria naturally will develop more readily if unsanitary conditions are present, but it certainly will not develop if the *Bacillus diphtheriæ* is absent. The same is true of other diseases, and consequently when we come to consider such a decidedly infectious disease as foul brood and learn the facts about it which such men as Cheshire have told us we naturally come to the same conclusion. If I were to maintain that a Carniolan queen might lay an egg which would develop into a humble bee, bee men would be inclined to think that not only my bee knowledge, but also my scientific knowledge, was at fault; but yet in all the bee journals I find many prominent bee keepers maintaining that an ordinary microbe which produces putrefaction may become metamorphosed into the specific cause of foul brood. It it is easy enough, however, to combat such an opinion upon a priori grounds, but not quite so easy to offer convincing proof.

In order to do this I thought it worth while to try some experiments. With this end in view I obtained some comb containing chilled brood and endeavored to isolate *Bacillus alvei* from it, but without success.

There were plenty of other bacteria, but none which presented the well-marked morphological characters peculiar to *Bacillus alvei*. Again I had sent to the laboratory a piece of perfectly healthy comb. I killed the brood by chilling. Then I infected some of the cells from a pure culture of *Bacillus alvei*. I allowed all the chilled brood to putrefy in a moist chamber for two weeks, at the end of which time I obtained *Bacillus alvei* again from the cells which had been artificially infected, but could find no traces of it in the other cells. I left this comb in a moist chamber for several months and again examined, but with the same results. In the cells in which *Bacillus alvei* had been placed it was still to be found; in the others it was not present.

It seems to me that an experiment such as the above conclusively shows that there is a distinct difference between foul brood and ordinary putrefaction.

In considering the subject of the vitality of *Bacillus alvei* the first question which naturally arises is its power to resist heat. We know that bacilli which produce spores and those which do not stand in entirely different positions in this regard. The sporeless bacillus is destroyed at a much lower temperature than one which contains spores. Consequently in considering the question of the vitality of *Bacillus alvei*, which produces spores very quickly and easily, we may confine our attention entirely to the vitality of the spore.

This is of special interest, as the question has been repeatedly raised whether it is dangerous to use comb foundation made from foul-broody wax. Does the temperature to which the wax is raised in the manufacture of comb foundation sufficiently destroy the vitality of the spore? Can the spore germinate and infect the brood when once inclosed in the wax?

These questions have been raised by many careful thinkers among bee men, and certainly deserve attention. The second point ought to be considered first, since if surrounding a spore with a film of wax prevents its germination, we need pay no further attention to the question of heat. The crucial test of this

would naturally be, supply a healthy colony with comb foundation known to contain the spores and observe the result. This I had hoped to try with the assistance of your secretary, but other work came up which interfered with the carrying out of this experiment and consequently it had to be postponed until next year. However, I was able to perform one experiment which throws some light on the subject. Mr. Holtermann, the secretary of your union, sent me several pounds of very fine wax, such as is used for the manufacture of comb foundation. I cultivated the *Bacillus alvei* upon agar jelly until I had a large quantity of the bacilli containing spores; this was carefully scraped off the jelly and dried first in the air and then over sulphuric acid. The resulting grayish mass was pulverized with a sterilized pestle and mortar, and finally mixed thoroughly with the melted wax kept at a temperature sufficiently low to prevent the immediate destruction of the spores by heat. By this means an enormous number of spores were introduced into the wax. After stirring the wax for some time in order to insure a proper mixing it was allowed to cool. This, as you all know, takes some time when dealing with a considerable quantity. During the cooling I was careful not to disturb the wax.

After it had solidified I set out to discover if I could again obtain my bacillus from the infected wax. If it would germinate in the nutrient media, it certainly would in the bees, and that point was to a certain extent settled. Now I obtained the following results:

From the upper layers of the infected wax I was unable to obtain cultures of the *Bacillus alvei*, either by melting the wax in the nutrient jellies or by allowing particles of the unmelted wax to fall on the surface of these jellies.

From the under layers, however, the results were different; particles of wax placed on nutrient agar in an oven kept at 98° F. became surrounded in twenty-four hours with a luxuriant growth of *Bacillus alvei*. When the wax was melted into the agar or into beef tea, I also obtained the bacillus, consequently it looks as if the mere fact of enveloping the spores with a film of wax was not sufficient to prevent germination. I confess I can not understand how a spore could germinate when surrounded with a film of wax. Spores in germinating require moisture, and if a spore is completely embedded in wax, it can not obtain sufficient moisture to germinate; I would rather believe, therefore, that in this particular experiment the spores had not each an envelope of wax, but that many of them were partially free from wax. Now, if this was the case in my experiment, where I endeavored to make the incorporation of the spores in the wax as thorough as possible, I certainly think it may frequently be the case when foul-broody wax is used and no particular precaution taken. That even when spores are thoroughly surrounded by wax they may not be freed occasionally by the workers is a point which requires further elucidation and upon which I intend to try some experiments next year.

In looking through the bee journals, however, I find it everywhere maintained by foundation makers that they never knew of a case of foul brood originating from foul-broody wax; and I have yet to discover a well authenticated case where this has occurred. What explanation can we offer of this widespread opinion?

I explained to you above that I was unable to cultivate *Bacillus alvei* from the upper layer of the infected wax. Your secretary also sent me a small specimen of wax which he stated he knew to be from foul-broody comb. This I examined repeatedly for foul brood, but was able to obtain it only once. I think we must look to the physical conditions for an explanation of the freedom from infection through comb foundation. The difference in the specific gravity of the bacteria and of melted wax is so great that throughout the

process of manufacture the bacteria tend to fall to the bottom. The first refining of the wax must, of course, remove the greater quantity, and the vast majority of the remainder will settle to the bottom during the process of foundation manufacture. But that the simple process of mixing the infected material with the melted wax is not sufficient to prevent germination I think is shown by the results quoted above, where simple fragments of infected wax when placed on agar jelly gave rise to a culture of *Bacillus alvei*.

This question I hope to touch on again after I have had an opportunity of supplying healthy bees with foundation made from infected wax.

The other question is whether the temperature to which wax is raised during foundation making is sufficiently high to destroy the spores of foul brood. In order to decide this question there are several points to be noted. The first is the character of the heat. We know that moist heat will destroy bacteria and their spores much more quickly than dry heat, and Mr. Corneil, of Lindsay, has raised this point several times, claiming that the heat to which the bacteria are exposed in melting wax is not moist heat but dry heat, consequently we must heat to a high temperature and for a long time in order to destroy the spores. The point is undoubtedly well taken, and can only be settled by direct experiment. In order to determine the temperature at which the spores are destroyed in melted wax, I used a method that was first described by Koch. Sterilized silk threads were saturated with a beef-tea culture of *Bacillus alvei* in which there were large numbers of spores. These threads were then allowed to dry and in the dry state were preserved. These dried threads were introduced into the melted wax and allowed to remain in it for a definite time at a fixed temperature. At the end of that time the thread was introduced into the melted agar or into beef tea heated to the melting point of wax, and thoroughly shaken, so as to separate the wax as much as possible from the threads; then the culture medium was rapidly cooled, and the tubes placed in the ordinary cultivating oven kept at 98° F. If I obtained a growth of bacilli, I concluded that the threads had not been sufficiently heated in the wax; if I did not, I concluded that they had been sufficiently heated. The following are my results:

At 212° F. (100° C.):

For one-quarter of an hour: Growth.
For one-half an hour: Growth.
For one hour: Growth.
For one hour and a half: Growth.
For two hours: Growth.
For two hours and a half: No growth.

At 194° F. (90° C.):

For one-half hour: Growth.
For one hour: Growth.
For two hours: Growth.
For three hours: No growth.
For four hours: No Growth.

On the other hand, a temperature of 122° F. (50° C.) did not destroy the spores in twenty-four hours.

I have repeated these experiments several times with the same results, so that I would conclude that to destroy the foul brood in wax it is necessary to heat to a temperature of at least 194° F. for at least three hours. Now the question arises, does this take place during the process of manufacture of comb foundation? In order to get as much data as possible on the subject I wrote to Mr. Larrabee, of Michigan Agricultural College, as he had kindly offered me

any assistance in his power. He applied to two prominent foundation makers for information. From their replies it is apparent that, for a short time at any rate, during the refining and purifying of the wax it reaches a temperature quite at or near 212° F. During sheeting, however, it apparently does not reach a temperature much above the melting point, say 175° F. They both seemed to agree that steam heat for too long a time injures the quality of the wax.

In the American Bee Journal, 1891, page 470, we find some statements on the subject in a reply by two prominent foundation makers to an article by Mr. Corneil upon the dangers of infected comb foundation. One of them, Mr. Dadant, states that in refining it is heated for some time at 212° F. and is kept liquid for twenty-four hours. The other, Mr. M. H. Hunt, states that it is kept at the boiling point for six or seven hours. If these are the actual temperatures reached during foundation making, I am inclined to think there is little danger from foul brood in that direction.

I thought it possible that the whole question could be settled by introducing a certain amount of some disinfectant—say beta naphthol—into the melted wax, but my results have not been satisfactory. Apparently even the introduction of 1 per cent beta naphthol into wax did not hasten materially the destruction of the spores. I was able to demonstrate the presence of living spores in wax containing 1 per cent beta naphthol and heated for two hours to 194° F.

From all these facts, and taking into consideration also the physical fact of the settling of the bacilli to the bottom, I should think that with reasonable care in the preparation of comb foundation the dangers of infection from this source would be slight. But that the spores may germinate after being mixed with the wax, I think I have shown.

Why the spores of the *Bacillus alvei* are killed so quickly in the melted wax I am not able to explain, but it may be due to the fact that the wax itself when heated to such a temperature has an antiseptic value. That the spores resist other antiseptics as strongly as do the spores of anthrax I have proved by testing.

Cheshire and others recommend a solution of 2 per cent carbolic acid for disinfecting the hive after removing infected comb, but on actual experiment with the infected silk threads I have found that 2 per cent carbolic acid did not kill the spores in six days. These results are similar to those obtained by Koch for the spores of anthrax, and show that 2 per cent carbolic acid can not be relied on to destroy the spores. However, the question of the value of antiseptics I will take up more in detail later on in this paper.

I would like to say a word or two now on the methods of treating the disease. There are practically two methods: first, the starvation method; second, the method of medicated sirup. Mr. McEvoy's method of treatment, it seems to me, is practically a modification of the starvation method. The first method is widely used both here and in the United States, whilst in England and Europe generally the second method is adhered to.

Considering the vitality of the spores of foul brood, it would seem at first sight useless to try any process which did not recognize as its foundation the destruction of the germ. I find, however, that many prominent bee keepers who have had practical experience with the method of starvation, or Mr. McEvoy's method, accept it as successful. I have not had an opportunity to examine colonies which have been cured in this manner, and so can not say that the bacilli have disappeared. I hope next summer to test this question more fully. We may, however, examine into the rationale of the method. In conversation with Mr. Corneil, of Lindsay, he made a suggestion which may be quite familiar to you all, but which seems to me the only explana-

tion. That suggestion was that either starvation or comb building carried the infected nurses past the period at which they act as nurses and gave them a chance to rid their intestines of the germ. If this is combined with a removal to absolutely clean hives, with new foundation, it may succeed, but I must say that absolute cleanliness in this respect must be insisted on. As I said above, I have not had any opportunity of investigating the results of these methods practically, and so can not speak with certainty.

The fact of the presence of the bacilli in the workers and in the queen bears, to a certain extent, upon this question. Cheshire and others make the statement that the bacilli are found in the intestines of the workers and in the ovaries of the queens. My own experience confirms this. I have found them repeatedly in the workers, and in five queens from infected hives I succeeded in obtaining the bacillus from the ovaries of three. That they are not always present in the ovaries of the queens from diseased colonies is certain; their presence there is apparently accidental. For instance, in the case of one last year's queens in a hive rather badly diseased I was unable to find the bacillus, whilst in a six weeks' queen from a hive in which there were only a few diseased cells I succeeded in finding it.

Cheshire's statement that he found a bacillus in an egg of an infected queen seems to me to require confirmation. I have not been able to find the eggs infected myself, but it is a question which would require very long and careful investigation before one could be able to deny or confirm such a statement.

In the second method of treatment by medication I do not think that an absolute destruction of the spores takes place, any more than in the starvation method. As I have shown above, 2 per cent carbolic acid was not sufficiently strong to destroy the spores, consequently it is not likely that 0.2 per cent (1 part in 500) would be strong enough. I tried 0.2 per cent, but found it quite unsuccessful. Its action then must have another explanation. To test this I made up a sterilized beef broth containing 1 per 500 of carbolic acid, and in it placed my infected silk threads. I found that there was no indication of growth. These threads were then taken out and placed in ordinary sterilized beef broth, and I obtained a luxuriant growth, i. e., the 0.2 per cent carbolic acid in the culture fluid, although it did not destroy the spores, prevented their germination. That, then, is the explanation of the value of carbolated sirup in the treatment of foul brood, it prevents the germination of the spores. The bee journals contain numerous examples of cases where carbolated sirup produced an improvement, but as soon as it was stopped there was a relapse. It is evident that here again, as in the starvation process, there must be combined an extremely thorough cleaning up, so that the best possible results may be obtained from the treatment. Medicated sirup does not destroy the spores, it simply prevents their development and gives the bees a chance to rid themselves of the infection, and in that respect I certainly think resembles the starvation process. Its advantage over that is that it can be carried on for a longer time.

In the course of these experiments I tried another substance which has been much used since Lortet's work on the subject, viz, beta naphthol. I do not think myself, from recent work on this substance, that beta naphthol should be ranked very high as an antiseptic, mainly on account of its insolubility in water. I found, however, that a beef broth containing 1 per 1,000 beta naphthol would not allow spores of *Bacillus alvei* to germinate, and consequently had an equal value with 1 per 500 of carbolic acid. It has an advantage over carbolic acid on account of the disagreeable taste of the latter, and I think would be more acceptable to the bees.

Salicylic acid in sirup has apparently the same effect, but I would not recommend the addition of borax, as Behring has shown that borax lowers considerably the antiseptic value of salicylic acid.

I tested also formic acid in the same way, but my results so far have not been satisfactory, owing to the uncertain strength of my sample of formic acid. I prefer to reserve a report upon it and other substances which I wish to try until later.

Mercuric chloride I have not tested, as I do not think it wise to use it around the hive. The idea of using a 1 per 1,000 solution to spray the diseased combs, as suggested sometimes, is, I think, absurd, and would be a rather serious operation for any living brood.

You will see that I consider all these methods of treatment do not in themselves necessarily presuppose the destruction of the spores, but depend upon the fact that for a longer or shorter period the spores are prevented from germinating, and in this period they are eliminated from the infected bees. Whether the vitality of the bees themselves has an effect upon the elimination or destruction of the spores is a point which would be extremely interesting, but one on which at present we have no definite information. From the results of bacteriological work on other diseases we know that the animal body is engaged in a constant warfare with the disease germs which may be introduced, and this also may be the case in foul brood. Much more extended investigations, however, would be necessary to prove this. It is much safer for apiarists to accept the possibility of a recurrence of the disease after a course of treatment, owing to the lodgment somewhere of some of the spores of *Bacillus alvei*, and by care and cleanliness remove this possibility. To do this the hives and frames in which a foul broody colony has lived must be sterilized, and this may be done in various ways. For the sterilization of material by disinfectants there was a tendency formerly among bacteriologists to run to such disinfectants as corrosive sublimate, carbolic acid, etc., but later work has shown that there are a number of common chemicals which will act just as well, or perhaps better. Corrosive sublimate has lost much of its reputation as a disinfectant within the last few years, and carbolic has been shown to be not nearly so powerful as at first supposed.

For cleaning hives and frames which are suspected to contain the spores of foul brood a hot 10 per cent solution of soft soap is perhaps as effectual as any that can be recommended. A good strong solution of washing soda, when hot, is also very active, destroying the spores in a few minutes. Both these are certainly better than 5 per cent carbolic for disinfecting the hives and frames, as their cleansing properties are so much better than it, and Behring has shown that 5 per cent carbolic requires at least three hours at blood heat to destroy the spores of anthrax. In case the soap or the washing soda is used, however, it must be used as hot as possible. Of course anything which is of no value should be burned.

I trust that in this paper I have thrown a little light upon some of the facts in connection with the disease of foul brood, but, as I stated in the beginning, I reserve the privilege of submitting to you at a future meeting the results of next summer's work.

Before closing I desire to express my thanks to your able secretary, Mr. Holtermann, for the assistance which he has given me, and also to Mr. Corneil, of Lindsay, for advice and for the use of volumes of all the principal bee journals, which he has supplied me with; also to Mr. Larrabee, of Michigan Agricultural College, in connection with the subject of comb foundation.

The next investigation to be considered is that by Prof. F. C. Harrison, professor of bacteriology of the Ontario Agricultural College. Previous to this Dr. William R. Howard published a paper on the subject, but this can be discussed better at a later time. In the paper by Professor Harrison, previously mentioned, the author gives a detailed description of the bacillus with which he worked. The description is as follows:

THE ORGANISM.

Bacillus alvei, Cheshire and W. Cheyne, 1885, from the larvæ of bees suffering from the disease known as foul brood, la loque (Fr.), and faul brut (Ger.).

Morphological characteristics.—In form the organism is a slender bacillus, with ends slightly pointed and rounded. "In the larval juices it is about $\frac{1}{7000}$ of an inch in length and $\frac{1}{20500}$ in breadth. On agar the bacilli vary considerably in size, averaging $\frac{1}{7260}$ inch, some small as $\frac{1}{10000}$ inch, and others as large as $\frac{1}{3000}$ inch. When they have attained the latter size, division of the rod seems to begin. They are always somewhat pointed at their ends. Their average breadth is $\frac{1}{300000}$ inch, ranging from $\frac{1}{35000}$ to $\frac{1}{25000}$ inch (Cheshire and W. Cheyne). Klamann (Bienenwirtschaftliches Centralblatt, Hannover, 1888, pts. 18 and 19) states that a clear space often appears in bacilli with pointed ends. From agar cultures twenty-four hours old, at 37° C., the bacilli average 4 μ in length and 1.0 μ in breadth. On gelatine cultures, grown at 22° C., they are somewhat shorter. They grow singly, but occasionally form chains of various length.

Stains.—With the ordinary aniline stains the bacilli colour rather badly (Eisenberg, Bakteriologische Diagnostik, Hamburg, 1891, p. 298, and Klamann, Bienenwirtschaftliches Centralblatt, Hannover, 1888, pts. 18 and 19). The best stains are methylene blue and methyl violet. The bacilli accept Gram's stain, but the spores are not colored by it. I find the most satisfactory stain in methyl violet.

Capsule.—No capsule has been demonstrated by Welch's method.

Flagella.—The bacilli are actively motile and possess a single flagellum at one pole. The motility of the bacillus is quite pronounced in fresh cultures obtained from bouillon, agar, and gelatine. The flagella stain by Pitfield's, Loeffler's, and Van Ermegen's method.

Spore formation.—Spores are formed by the bacillus, and are large oval bodies averaging in length $\frac{1}{12000}$ inch, and in breadth $\frac{1}{23700}$ of an inch. On agar the spores are arranged in long rows, side by side, and are greater in diameter than the cells from which they are derived. The earliest appearance of spore formation takes place in forty-one hours, at 36° C. (Cheyne), but in some cases it is even sooner. The spores are formed in the center of the rod, and the formation occurs as follows: The rod begins to swell and become spindle-shaped. Occasionally the swelling is more marked at one end than in the center. The spindle-shape increases in size, and the center of the swelling gradually ceases to take the stain. The capsule of the spore is apparently formed within the rod and is not merely the outer part of the rod. In three or four hours the rod is seen to have almost or completely disappeared, although parts of the faint outline of the ordinary bacillus may be noticed.

Germination of spores.—Under favorable conditions the beginning of the germination of the spores takes place in about three hours. The spore loses its oval

shape, becomes elongated, and is soon seen to burst through the spore capsule. It then presents the appearance of a short rod, with a pale envelope embracing one end. The rod gradually leaves the spore capsule, and then goes on multiplying as a full-grown bacillus. According to Eisenberg (Bakteriologische Diagnostik, Hamburg, 1891, p. 298) the spores are decolorized by the tubercle bacilli stain, but preparations may be obtained by using the Ziehl-Neilsen stain and alcohol for decolorization. The spores also stain by the method of Neisser.

Polymorphism.—Variations in size and shape may be brought about by growth in acid media, or in media containing different sugars. These variations occur also in the same culture, subjected to exactly similar conditions of growth.

Involution forms.—Abnormal forms are especially abundant when the bacillus is grown on blood serum; peculiar Y-like forms and clubbed shapes are of common occurrence, and relatively few spores are found.

BIOLOGICAL CHARACTERS.

Bouillon.—"In meat infusion at the temperature of the body they grow rapidly, causing muddiness and, after a few days, a slight but not tenacious scum" (Cheshire and W. Cheyne). In bouillon, with a reaction of +0.08 (Report of Convention of American Bacteriologists, Journal American Public Health Association, Vol. XXIII, 1898), at 37° C., there is a slight turbidity in fourteen hours, especially noticeable when the tube is shaken. In twenty-four hours, the liquid is uniformly turbid, with a very fine sediment. In forty-eight hours the turbidity increases and a pellicle commences to form. Reaction of the culture at this time, +0.07. After ninety-six hours the broth is clear, with a pellicle, white, rather massive, and somewhat tenacious. There is also much sediment. Reaction, after ten days' growth, neutral.

Glycerine bouillon.—Media with original reaction of +0.08. At 37° C. the bouillon becomes slightly turbid in twelve hours and quite turbid in twenty-four, with a fine, whitish pellicle on surface, which does not extend to the sides of the tube. If the culture is shaken, the pellicle deposits in flaky masses. The reaction is +1.2. In thirty-six hours the turbidity clears, leaving the media bright, with a smooth, thin, tenacious, and white pellicle on the surface. In many cases the pellice becomes very wrinkled and greasy looking. At the end of eight days the reaction is +2.2, and the bouillon is several shades darker in color, but quite clear. The reaction after fourteen day's growth is +4.2. At 22° C. the same changes occur, but growth is slower. The bacilli are relatively less numerous than in bouillon and are slightly shorter and thicker.

Glucose bouillon.—With a reaction of +2, at 37° C., the broth is more turbid than plain bouillon after fourteen hours' growth; and in twenty-four hours the sediment is heavy and turbidity very marked, but no pellicle. In forty-eight hours the media is opaque and cloudy, and the pellicle is beginning to form. In ninety-six hours the broth is less cloudy, but the sediment is heavier, and a white, thick pellicle is formed. It is often wrinkled, but not quite so much so as that on the glycerine broth. Reaction of broth after ten days' growth, +4.6. The bacilli are occasionally clubbed, and Y-like forms may occur. They average 5 μ in length and may be slightly curved.

Lactose bouillon.—With a reaction of +1.06, at 37° C., the growth resembles that of plain bouillon for the first twenty-four hours; but at the end of forty-eight hours, it is more turbid. In ninety-six hours, a tenacious pellicle forms, less massive than that on glucose broth. Reaction after ten days' growth, +2.4. The bacilli average 3.5 μ in length.

Saccharose bouillon.—With a reaction of +1, at 37° C., the turbidity and sediment are heavier than any of the other bouillons. In forty-eight hours the

broth is quite opaque and whitish looking. A heavy sediment is then present, and pellicle formation is just beginning. In ninety-six hours the cloudiness is about the same, but there is an increase of sediment, and the pellicle is thin and membranous. Reaction of media after ten days' growth, +4.04. The bacilli average 5 μ in length.

Gelatine plates.—At 22° C., in twenty-four to thirty-six hours, the colonies are small, round, oval, or lozenge-shaped, with peculiar projections or shoots from one end of the colony, giving it a pear-shaped or tadpole-like appearance, according to the amount of development of the projection. In many cases several of these outgrowths occur from different portions of the colony. By placing a cover glass on the surface of the gelatine and using objective 7, the bacilli may be seen moving around and around the colony and to and fro along the projections. At the end of forty-eight hours the colonies are larger. Fine processes or projections are shooting out into the gelatine in all directions, forming peculiar figures in circles or club-like forms. "It is impossible," says Cheyne, "to give a proper idea of the appearance of the growth. The forms assumed are the most beautiful shaped I have ever seen; but they are very numerous, always retaining the tendency to form curves and circles." After a time the gelatine is liquefied and the beautiful appearance of the colony is destroyed by the liquefaction of the gelatin.

These peculiar shaped colonies are most typical when the germ is taken from the diseased larvæ. After prolonged cultivation on various kinds of media, there is a tendency for the colonies to become round, and the peculiar branching forms are not seen in such numbers. The composition of the gelatine also seems to make a difference in the appearance of the colonies. In gelatine containing 12 per cent gelatine the processes are not so long. The same effect may be brought about by using more peptone in the composition of the media.

Gelatine tubes.—In stick cultures at 20° C. growth occurs all along the line of puncture. On the surface delicate branching or ramifying growth occurs in three days. These outgrowths soon run together and the gelatine is liquefied, first around the line of puncture, and in five days extends over the whole surface. The growth in the depth of the gelatine occurs as a whitish streak all along the needle track, and from this numerous shoots and growths branch out into the gelatine in all directions, giving a haziness to the appearance of the gelatine, which then begins to liquefy. If the inoculation is a heavy one, the shoots are coarse and may have club-shaped extremities, and from these swollen ends fresh shoots may start. Cheyne obtained the most characteristic growth in gelatine containing 3 per cent of peptone as well as 10 per cent gelatine. The whole tube is liquefied in from two to four weeks' growth. The liquid becomes yellowish in color and gives off a peculiar odor. Klamann states that in gelatin acidified with lactic acid the growth is slow and long threads are formed.

Gelatine streak cultures.—In gelatine streak cultures the appearance is very similar to what one sees in stick cultures. The bacilli first grow along the line of inoculation, and then throw out shoots into the surrounding gelatine, producing the appearance noted in the stick culture. The bacilli move to and fro along the channels of liquefied gelatine.

Agar plates.—On agar plates at 37° C. the colonies at the end of eight hours are small and burr-like, with spines protruding in all directions, giving the colony the appearance of a sea urchin. In some cases the projections are from one side or end. At the end of twelve hours the colonies have well-defined projections, visible to the naked eye. The colonies in the depths of the agar are more spiny, the processes being much shorter. On agar plates

streaked with a light inoculation most beautiful forms occur. The growth of the bacilli spreads over the surface and branches repeatedly, giving the appearance of seaweed. This appearance is distinctly characteristic; and as the growth is very rapid, this method commends itself for making a quick diagnosis of the presence of the bacillus in larvæ supposed to be diseased.

Potato cultures.—On potatoes the growth differs considerably, according to the reaction and age of the potato. Sometimes a brownish wrinkled growth forms, which gives off a peculiar odor; at other times a dryish yellow layer appears. "The bacilli grow very slowly indeed at 20° C." (Cheyne, Journal of the Royal Microscopical Society, 1885, p. 381). Even at 37° C. they grow slowly.

Milk.—In milk at 37° C. coagulation of the casein occurs in three days. The milk becomes yellowish and gives off a characteristic odor. After several weeks' growth the curd is digested and a whey-like fluid remains.

Blood serum.—On blood serum at 37° C. the growth is rather slow and polymorphic forms are common. "Very long filaments are formed" (Cheshire and W. Cheyne, Journal of the Royal Microscopical Society, 1885, p. 381). These long forms may be from five to ten times as long as the average bacillus growing on gelatine, and consists of single cells. The filaments are often wavy or twisted and of unequal thickness. The extremities of the long, bent rods are often clubbed; and Y-like forms are numerous. Spores are formed very sparingly, and the blood serum is liquefied.

Synthetic media (Uschinsky).—In Uschinsky's medium no growth occurs; but if the medium is neutralized, good growth ensues. The bacilli occur in threads and a pellicle is formed.

Dunham's solution.—The bacilli are small when grown in this solution. No threads form, but there is a slight indol reaction after nine days' growth.

Relation to free oxygen.—Cheyne states that the germs grow most rapidly on the surface of agar and arrange themselves side by side; and they produce spores in this position after a few days' growth. Eisenberg (Bakteriologische Diagnostik, Hamburg, 1891, p. 298) says nothing under the head of aerobiosis. Howard (Foul Brood: Its Natural History and Rational Treatment, Chicago, 1894) writes that, "It grows best under anaerobic conditions; is a facultative aerobe; grows under the mica plate, and in the presence of oxygen the growth is slight and slow." Howard also states that under anaerobic conditions it emits a foul odor resembling that of foul brood. It will be thus seen that Cheyne and Howard do not agree on this point. The former author also says that the characteristic odor is given off under aerobic conditions, whilst Howard states that this smell is emitted under anaerobic conditions. Further, Cheyne states that the bacilli grow with great rapidity on the surface of agar, whereas Howard obtains his best growth under the mica plate, which does not give complete anaerobiosis. Howard's conclusions are thus at variance with Cheyne's, and my own results fully corroborate those of the latter author.

Howard states that the vitality of the spores of *B. alvei* is destroyed when exposed to atmospheric air from twenty-four to thirty-six hours. In making his experiments he took sterilized road dust and mixed it with the dry foul-brood masses from several cells, which were previously dissolved in distilled water. The mixture was worked dry and spread on sheets of paper, and trial cultures were made immediately and at intervals of every twelve hours for three days; and, according to his results, no growth occurred after thirty-six hours. In giving these results, Howard does not state whether he exposed the spores to sunlight or diffused light; nor does he mention the age of the dry foul-brood masses, which he used from several cells. These are points of considerable

importance, for, as everyone knows, the disinfecting power of direct sunlight is much greater than diffused light, and the vitality of the spores from foul-brood masses of different ages varies considerably. This, I may add, has been clearly shown by some of my experiments, subsequently described. In my experiments the spores obtained from a pure culture on the surface of agar were spread on cover glasses and placed in a glass chamber, so arranged that a current of air was constantly circulating over them. This chamber was exposed to the ordinary light of a room with six large windows, and a cover glass was taken out every twenty-four hours and tested, to see if the spores would grow. This experiment was continued for one month, and at the end of that time the spores still germinated rapidly. In another experiment, spores spread on cover glasses were exposed to a very diffused light, simulating, as far as possible, the amount of light which would enter a hive. Cover glasses were taken out from time to time and transferred to agar, in order to ascertain if the spores were alive or not. The experiment was begun two years and four months ago, and from the last cover glass taken and placed upon the surface of an agar plate a copious and typical growth of *B. alvei* was obtained. Further, thin strips of filter paper, plunged into a bouillon culture and allowed to dry, were threaded on a wire suspended in a wire basket and so exposed that the air could freely circulate around them in the ordinary light of a room. Trial cultures were made at intervals, and at the expiration of six months the spores from the paper germinated when strips were placed on the surface of agar.

Again, a drop of bouillon containing spores was placed in a sterile tube and allowed to dry; and at the expiration of one hundred and twenty-four hours (thirty-six of which were in sunlight at a temperature varying from 30° to 37° C.) sterile bouillon was added. The tubes were then placed in the incubator, and in less than twenty-four hours a good growth of the germs had taken place.

From these experiments it will be seen that the results are directly at variance with Howard's statement, as they go to show that the vitality of the spores of *B. alvei* is not destroyed by exposure to atmospheric air, with or without sunlight, for even a much longer time than twenty-four to thirty-six hours.

With regard to the aerobiosis of this bacillus, good growth has been obtained in an atmosphere of hydrogen by Novy's method. Buchner's method also gave good results. The growths in the various media are very similar to those produced under aerobic conditions, but with this difference, that the surface growths are, as a rule, whiter in the hydrogen atmosphere. In illuminating gas (water gas) no growth occurred, but the spores were not destroyed by the action of the gas; for when the gas was let out of the Novy jar, good growth ensued on all cultures. In acetylene gas, a restricted growth occurred. In fermentation tubes growth occurred both in the open and in the closed arm of the tubes. No gas was formed, the bouillon in the closed arm was uniformly turbid. Thus *B. alvei* is a facultative anaerobe.

Production of alkali.—In ordinary bouillon a slight amount of ammonia is formed. Control bouillon did not give the Nessler test. In glycerine and the sugar bouillons, there is no trace of ammonia. Cheyne's cultures are faintly alkaline, both before and after inoculation in meat infusion. Klamann states that ammonia is produced.

Acids formed.—A varying amount of acid is formed. All the sugar bouillons give an acid reaction.

Formation of pigment.—On potatoes a yellowish growth is produced; on all other media, the surface growth is white.

Development of odors.—Cheyne states that gelatine cultures give off an

odor of stale, but not ammoniacal urine, or what may be better described as a shrimpy smell; and this peculiar odor has been found by Cheshire to be distinctive of diseased larvæ. Klamann and Howard both state that a peculiar odor resembling that of the diseased larvæ may be noticed in artificial cultures.

The effects of desiccation.—I have already noticed, under the head of "Relation to free oxygen," that the spores of *B. alvei* have considerable vitality in withstanding desiccation. My experiments prove conclusively that the spores are extremely hard to kill by desiccation and in this respect resemble those of anthrax, which are known to resist thorough desiccation for a number of years. One experiment which showed this characteristic was as follows: An agar plate completely covered with a typical growth of *B. alvei* was allowed to dry out completely, and was left exposed to the ordinary light of the room for seven months, and at the end of that time, a portion of the film was scraped off with a knife, placed on suitable medium and incubated, with the result that a typical growth immediately ensued.

Spores on cover glasses were exposed to September sunlight (latitude 43°) for varying periods of time, and growth occurred after four, six, and seven hours' exposure. The age of the spores varied from five days to eighteen months; and spores three months old were not killed by seven hours' exposure.

From the symptoms given in this paper the disease with which Professor Harrison worked was doubtless American foul brood. From the discussion of geographical distribution this is also evident, for he says: "I have examined diseased larvæ from Canada, from Europe, * * * Cuba, and thirteen States of the Union, ranging from New York to California and from Michigan to Florida." American foul brood is thus widely distributed, but from all these specimens Professor Harrison obtained a bacillus which he called *Bacillus alvei*. Since we now know that *Bacillus alvei* is found in European foul brood and not in American foul brood, it is evident enough that the germ must have been another bacillus. European foul brood, as far as the author is able to learn, is not found in Canada nor Cuba, and, although now found in several States in the northeastern United States and spreading, is not, as Professor Harrison would have us believe, widely distributed in the United States.

How can this be accounted for? The only way open seems to be in the identification of the bacillus. I do not feel qualified to pass judgment on the accuracy of the description of Professor Harrison, but the matter has been referred to Doctor White, and he assures me that the description just quoted fits the bacillus which is described as *Bacillus A* as well as it does *Bacillus alvei*. If this is true, we can only conclude that Professor Harrison, not knowing of the existence of two diseases, made a serious error in his identification. In no place does he speak of any difficulty in obtaining cultures from American foul brood. For comparison, Doctor White's description of *Bacillus A* (possibly *B. mesentericus*) is here quoted.

Bacillus A.

(*B. mesentericus?*)

Occurrence.—Found very frequently on combs, on scrapings from hives, and on the bodies of bees, both diseased and healthy.

Gelatin colonies.—Very young colonies show irregular edges, but very soon liquefaction takes place and the colony gives rise to a circular liquefied area, covered with a gray membrane, which later turns brown.

Agar colonies.—Superficial colonies present a very irregular margin consisting of outgrowths taking place in curves. Deep colonies show a filamentous growth having a moss-like appearance.

Morphology.—In the living condition the bacilli appear clear and often granular, arranged singly, in pairs, and in chains. The flagella are distributed over the body. The rods measure from 3μ to 4μ in length, and from 0.9μ to 1.2μ in thickness.

Motility.—The bacilli are only moderately motile.

Spores.—Spores are formed in the middle of the rod.

Gram's stain.—The bacilli take Gram's stain.

Oxygen requirements.—Aërobic and facultatively anaërobic.

Bouillon.—Luxuriant growth in 24 hours, with cloudiness of medium; a gray flocculent membrane is present. Later, the membrane sinks and the medium clears, leaving a heavy, white, flocculent sediment, with a growth of the organisms adhering to the glass at the surface of the medium. Reaction alkaline.

Glucose.—Luxuriant growth takes place in the bulb, with a moderate, flocculent growth in closed arm. The gradual settling of the organisms causes a heavy white sediment to form in the bend of the tube. The reaction is at first slightly acid, but subsequently becomes alkaline. No gas is formed.

Lactose.—Reaction alkaline.

Saccharose.—Reaction alkaline.

Levulose.—Reaction acid.

Maltose.—Reaction acid.

Mannite.—Reaction alkaline.

Potato water.—Reaction alkaline.

Agar slant.—A luxuriant growth takes place on this medium. The growth gradually increases to a moist, glistening one, being then friable and of a grayish brown color.

Serum.—A luxuriant, brownish, glistening, friable growth spreads over the entire surface. No liquefaction is observed.

Potato.—An abundant fleshy growth of a brown color spreads over the entire surface. The water supports a heavy growth. The potato is slightly discolored.

Milk.—Precipitation takes place rapidly, followed by a gradual digestion of the casein, the medium changing from the top downward to a translucent liquid, becoming at last semitransparent and viscid.

Litmus milk.—Precipitation of the casein takes place usually within 24 hours, followed by a gradual peptonization. Reduction of the litmus occurs rapidly, leaving the medium slightly brown; later the blue color will return on exposing the milk to the air by shaking. Reaction alkaline.

Gelatin.—An abundant growth takes place with rapid, infundibuliform liquefaction. A heavy, white, friable membrane is formed on the surface of the liquefied medium. A flocculent sediment lies at the bottom of the clear liquefied portion.

Acid agar.—Growth takes place.

Indol.—None has been observed.

Nitrate.—Reduction to nitrite is positive.

Dr. William R. Howard, of Fort Worth University, Fort Worth, Tex., has published several papers on the bacteriology of bee diseases. In a paper published in 1894 (York Publishing Company, Chicago) he attributed "foul brood" to *Bacillus alvei*. Evidently he was dealing with American foul brood, and we now know that *Bacillus larvæ* is present in that disease.

The same author undertook to determine the cause of pickle brood and described a specific fungus, *Aspergillus pollinis*. No investigator has since been able to find any such fungus in similar specimens.

In 1900 (Gleanings in Bee Culture, p. 121) this author published an account of some brief and entirely inadequate investigations made on what he chose to call "New York Bee Disease, or Black Brood." A specific organism, *Bacillus milii*, is described, but the view is expressed that this is modified, perhaps, by *Bacillus thoracis*. During the investigations of the Department of Agriculture it has been learned from whom Doctor Howard got his specimens, and the same men have furnished specimens which they declare to be of the same diseased condition as those furnished Doctor Howard. These, however, contain *Bacillus alvei*, and the disease is the same as that described by Cheyne, now named European foul brood.

It is most unfortunate for Doctor Howard that in not a single point have his descriptions been verified. Certainly it would seem unwise in him to put out the names *Bacillus milii* and *Bacillus thoracis* as new species without descriptions and after so short an investigation. We can not, therefore, sympathize very much with him when his views are overthrown.

The American bee journals and text-books on apiculture have until recently contained statements to the effect that *Bacillus alvei* is the cause of the disease which has been almost universally called "foul brood." This is due not only to the publications of Mackenzie, Harrison, and Howard, but very largely also to the attempt to determine *Bacillus alvei* by microscopic examination. The best-known case of this is probably the examination of diseased brood made by Mr. Thomas William Cowan, editor of the British Bee Journal. On a visit to Medina, Ohio, Mr. Cowan was shown a sample of diseased brood, and after a microscopic examination he announced that he found *Bacillus alvei*, and that the diseased condition is identical with that found in England. That this ropy type (for such it was) is found in England can not be doubted, but that the germs which Mr. Cowan saw were *Bacillus alvei* may well be doubted. I have taken particular pains to ask Mr. E. R. Root, who was present, whether Mr. Cowan made a cultural examination, and was assured that the microscopic examination was the only one made.

The announcement of this examination in Gleanings in Bee Cul-

ture and the A B C of Bee Culture, coupled with the excellent reputation of Mr. Cowan, made this appear convincing to American bee keepers. It must be remembered, however, that at that time no one had questioned the presence of *Bacillus alvei* in American foul brood and on finding bacilli the conclusion that they were *Bacillus alvei* was natural, even though erroneous.

Mr. Edward Bertrand, in his book "Conduite du Rucher," makes a similar announcement, stating that he and Mr. Cowan examined brood described as ropy and found *Bacillus alvei*.

[Mr. DADANT: I have received a letter from Mr. Bertrand. He informs me that they (Mr. Cowan and himself) had examined foul brood, but I know from the tone of the letter that no cultures were made.]

To indicate how much reliance may be placed in microscopic examinations in the absence of cultural tests, let me quote from Sternberg's Text-book of Bacteriology, 1901 edition, pages 13 and 14. It should be borne in mind that this refers to all microscopic examinations of bacteria and not specifically to bee diseases.

The bacteria are also classified according to their biological characters, and it will be necessary to consider the various modes of grouping them from different points of view other than that which relates to their form. This is the more important, inasmuch as we are not able to differentiate species by morphological characters alone. Thus, for example, there are among the spherical bacteria, or micrococci, numerous well-established species which the most expert microscopist could not differentiate by the use of the microscope alone; the same is true of the rod-shaped bacteria. The assumption often made by investigators who are not sufficiently impressed with this fact, that two micro-organisms from different sources, or even from the same source, are the same because stained preparations examined under the microscope look alike, has led to serious errors and to much confusion. As an example of what is meant, we may refer to the pus organisms. Before the introduction of Koch's "plate method" micrococci had been observed in the pus of acute abscesses. Some of these were grouped in chains—streptococci—and some were single, or in pairs, or in groups of four; but whether these were simply different modes of grouping in a single species, or whether the chain micrococci represented a distinct species, was not determined with certainty. That there were in fact four or more distinct species to be found in the pus of acute abscesses was not suspected until Rosenbach and Passet demonstrated that this is the case, and showed that not only is the streptococcus a distinct species, but that among the cocci not associated in chains there are three species which are to be distinguished from each other by their color when grown on the surface of a solid culture medium. One of these has a milk-white color, one is of a lemon-yellow color, while the third is a golden-yellow.

This brings us down to the work of Doctor White. His investigations were begun with Dr. V. A. Moore in 1902, and in January, 1903, a preliminary report was published. During the first year specimens of "black brood" were examined and to the surprise of the investigators *Bacillus alvei* was found in every case. Obviously,

then, they were working with the foul brood of Cheshire and Cheyne (European foul brood). In a second short paper by Doctor White a brief note is given concerning some work done on the "ropy type" of foul brood. He recognized that he was dealing with a disease the cause of which had not been described and the disease is called for the time "X brood" and the bacillus, *Bacillus X*. The final results of the investigation appear in Technical Series, No. 14, Bureau of Entomology, under the title "The Bacteria of the Apiary, with Special Reference to Bee Diseases." Doctor White's description of *Bacillus larvæ* is as follows:

BACILLUS LARVÆ.

Occurrence.—Constantly present in diseased brood from colonies affected with American foul brood.

Gelatin.—There is no growth.

Morphology.—It is a slender rod, having a tendency to form in chains. This is especially true when grown in bee-larvæ bouillon.

Motility.—The bacillus is rather sluggishly motile.

Spores.—Spore formation takes place. This can be observed best in the different stages of the disease and decay of the larvæ.

Oxygen requirements.—When Liborius's method is used, the best growth usually appears near to but not on the surface. After a few generations a surface growth may be obtained.

Bouillon.—There is no growth.

Glucose bouillon.—There is no growth.

Lactose.—There is no growth.

Saccharose.—There is no growth.

Agar plate.—There is no growth.

Bee-larvæ agar.—The inoculations must be made with the medium liquefied. The growth takes place near to but rarely on the surface. Cultures must pass through a few generations before a satisfactory surface growth can be secured.

Bee-larvæ agar slant.—On the surface of this medium a thin, gray, nonviscid growth takes place.

Glucose agar.—Slight growth has been observed in the medium. No gas is produced.

Potato.—There is no growth.

Milk.—There is no growth.

Litmus milk.—There is no growth.

Fermentation.—In bee-larvæ bouillon no gas is produced.

Indol.—There is no growth in sugar-free bouillon.

To summarize, then, *Bacillus alvei* is found universally in European foul brood; *Bacillus larvæ* in American foul brood. No specific micro-organisms have been found for the so-called pickle brood or paralysis. Knowledge of the two worst brood diseases is accurate enough to enable us to combat them by applying principles acquired by comparison with results of work with other micro-organisms.

That our knowledge is complete is far from true. Not only is there much to be learned which is of purely scientific interest, but points of the highest practical importance are yet undetermined.

[NOTE.—Several other papers of importance have been issued on this subject which were not discussed at the Inspectors' meeting. They, however, have an important bearing on this subject. Lambotte decided that *Bacillus mesentericus vulgatus* causes "foul brood," or, in other words, that *Bacillus alvei* is but a variety of *Bacillus mesentericus*. His work, however, is far from convincing. The principal point of interest in this regard is that he had great difficulty in getting a growth from the ropy type of foul brood (American foul brood) on ordinary media. Burri in 1904 published an account of his work and found *Bacillus alvei* in a few specimens from Switzerland (indicating that European foul brood is found there), but found another organism which grows with difficulty; the latter is undescribed and unnamed, and it is possible and probable that he worked with *Bacillus larvæ* White. Maassen (1906) found the same difficulty, isolating *Bacillus alvei* in only 13 specimens of diseased brood out of 112 received. He, too, found an organism which could be made to grow on ordinary media only with difficulty and called by him *Bacillus brandenburgiensis*. It is undescribed, so far as is known to the writer. He also claims to have found another organism, *Spirochæte apis* Maassen, but has not established any causal relationship.

These papers all tend to confirm the work of Doctor White. *Bacillus alvei* is not found in the ordinary ropy type of "foul brood," but another organism is; this is probably the *Bacillus larvæ* of Doctor White.

In the face of all these facts several prominent bee men of England are attempting to discredit all this work, the criticism, so far as is known to the writer, being based entirely on comparisons of literature and on an entire lack of investigation. They have, further, misread the papers issued by the Department of Agriculture on this subject. It seems entirely unnecessary, therefore, to review the criticism in detail.—E. F. P.]

EXISTENCE OF BOTH AMERICAN FOUL BROOD AND EUROPEAN FOUL BROOD IN THE SAME COLONY.

Mr. ATCHLEY. Do you think that both diseases, American foul brood and European foul brood, could exist in the same colony?

Doctor PHILLIPS. Reports are sometimes received that a colony is infected with both diseases at the same time, but this is contrary to the experience of those persons most conversant with these conditions. While it may be possible for a colony to have the infection of both diseases at the same time, it is not by any means the rule, and such cases are probably not authentically reported.

Both diseases are found in New York State. The inspectors have to treat both diseases and they treat both in the same way, but they have never found both diseases in the same colony.

GEOGRAPHICAL DISTRIBUTION OF AMERICAN FOUL BROOD.

Mr. DADANT. Is not the American foul brood spread more over the world?

Doctor PHILLIPS. It would seem so from the literature. It is found in almost every State of the Union, while European foul brood

is, as far as is known, found only in the States mentioned a while back.

Mr. DADANT. The American foul brood is characterized by the ropy condition. The French name for their common brood disease is "loque," meaning tatters, and this name therefore refers to what we call American foul brood.

Mr. COGGSHALL. Which of these diseases is the one found in Cuba?

Doctor PHILLIPS. That is the American foul brood. It has the typical ropy character.

EFFECT OF CLIMATE ON VIRULENCE.

Mr. L. F. JUNEAU (Colorado). I would like to ask if brood diseases are equally bad in all States or has climate anything to do with the virulence of the diseases?

Doctor PHILLIPS. Climate undoubtedly makes a great difference. The American foul brood of California is not anything like the same disease in the East. It is simply terrible in California. Mr. Rankin will tell us about that later in the day. It is the same disease, but its ravages are much worse.

Mr. JUNEAU. Mr. T. L. Thompson (Colorado) sent some pickle brood to Dr. W. R. Howard, and the latter called it "black brood," but said: "In your State it will not be so bad."

Doctor PHILLIPS. It was probably not European foul brood. That disease has not been found west of the Mississippi River.

ASSOCIATION OF INSPECTORS OF APIARIES.

It was decided that it would be well for the inspectors of apiaries of the various States to be organized in some way to bring about greater cooperation in the work. After discussing the question it was finally decided that Mr. N. E. France, inspector of apiaries for Wisconsin, should act as chairman of a committee on organization and appoint his own associates.

SECOND SESSION, HELD MONDAY AFTERNOON, NOVEMBER 12, 1906.

Doctor PHILLIPS. In the morning session we covered rather thoroughly the scientific side of investigations on bee diseases. That is, of course, important; but when it comes to the practical work on bee disease there are two subjects of much greater importance, namely, methods of treatment and legislation. We will first discuss the treatment of these two brood diseases and then take up the discussion, in so far as we can, of the laws now existing, with suggestions as to the form which a law should have to give the best results and the powers which should be given to the inspectors under the various conditions which may arise. I have, however, a paper that I wish to read first,

which was written by Mr. Charles Stewart, of Sammonsville, N. Y., one of the inspectors of that State. I had the pleasure and privilege of spending four weeks in the field with Mr. Stewart last spring, and I feel that I can say that if there are any good inspectors in the United States one of them is Mr. Charles Stewart. Mr. Stewart is exceedingly sorry that he can not be here, and he requested me to read this paper to you.

APIARY INSPECTION IN NEW YORK STATE.

By Chas. Stewart,
Inspector, Third District.

Brother Inspectors: It is with a feeling of regret that I write this paper, knowing that it will be impossible for me to be with you at what must be both a pleasant and profitable meeting.

It is hardly necessary for me to describe European foul brood nor to refer to its entrance into New York State, except to say that it was brought in some years ago by a shipment of bees from one of the Southern States, and just as we were feeling that we had nearly stamped it out and were masters of the situation we discovered that at least one if not two fresh importations had been made in a section of the State where no trouble of this kind formerly existed.

I wish to call your attention to the fact that no bee keeper can feel reasonably safe from infection until every State in the Union is under the surveillance of a keen-eyed inspector who knows every spot of disease in his jurisdiction and allows no bees to be shipped out of such territory. Had the inspectors of New York State not adopted this rule, disease would have spread not only all over our State, but to far distant points, as many, fearing the loss of their apiaries, were eager to sell at a sacrifice. In order not to make this rule a hardship to our people, we have made it a practice to find a buyer within the diseased territory competent to cure the disease and so keep our troubles within our own family.

I wish I had the power to paint to you in words the pathetic picture when four good men and true, who had been bee keepers from boyhood and had large interests to protect, took up this work. I have seen the faces of strong men blanch with fear or turn crimson with anger at the first visit of an inspector, and later, when their bees were saved and their product marketed, the young man sent back to college, their little children cared for, or perhaps the home saved, these same men with tears on their cheek would give one a hand clasp that was far more eloquent than words and possesses a value beyond gold. I question if there is an inspector present to-day who from a mere money point of view would not be better off if he had given

his entire time to his own business; yet I honor this American spirit you possess in that, having once started out to accomplish results, you refuse to turn back until the end is attained.

I hardly feel like posing as an instructor to this gathering of inspectors, but will call your attention to a few important points. A question often asked is, "How does the disease spread so rapidly?" I would answer, "By means of infected honey." No field bee from an infected colony goes out with its honey sack so empty of honey that it contains no germs, and on their return many bees mistake their hive and carry disease to their near neighbors in the same apiary, so that it is a common thing to find a badly infected colony and those in the same row infected in proportion to their distance from the source of contagion.

How the disease spread from yard to yard when no robbing took place was for a long time a puzzling question, until I found an apiary of black bees 3 miles from an apiary of golden Italians that were infected. In many colonies of the yard of blacks could be found a sprinkling of the golden Italians and in nearly every case these colonies showed traces of disease. Evidently bees are often driven by stress of weather or some other cause to seek shelter far from home, and thus disease may be spread.

We have found no bees immune from disease, yet some vigorous strains of Italians are nearly so. For years we have recommended the introduction of young Italian queens, but have warned the owner of an infected apiary not to depend on that alone, as it would prove disastrous in localities where the disease has just made its appearance and the bees are mostly black. This method will often prove very successful where European foul brood has existed for some time and lost much of its virulence, but, like the use of drugs, it is not a safe method for the inspector to advocate, while the shaking method has never failed us if done in a thorough manner. Colonies that are found to be diseased late in the season may be cured by taking away all their combs after brood rearing has ceased and giving them clean combs from a healthy colony, as any disease germs that are contained in the honey sack will have been eliminated long before brood rearing commences in the spring.

In conclusion I would say that to be successful as an inspector a man should not only be well versed in the management of bees and bee diseases, but he must be broad minded, even tempered, possess a liberal amount of tact and diplomacy, and be a shrewd judge of human nature. Yes, and even more, he should be able to win the confidence of others and share their burdens, and when the time draws nigh when the working tools of life shall drop from his nerveless clasp he may look back with satisfaction to a life well spent in the service of others.

Doctor PHILLIPS. When the notices were first sent out concerning this meeting I wrote to Mr. Fred A. Parker, of Lompoc, Cal., under the impression that he was inspector of Santa Barbara County. It seems that he has resigned and Santa Barbara County now has no inspector. He has, however, sent a paper, which will now be read:

AMERICAN FOUL BROOD ON THE PACIFIC COAST.

By FRED A. PARKER,
Former Inspector for Santa Barbara County, Cal.

During my term as inspector of apiaries for Santa Barbara County, Cal., in the year 1905, 4,073 colonies of bees were inspected. I discovered 47 cases of American foul brood and found 170 colonies not on movable frames. These were ordered transferred, and in some instances I did the work myself. Every case of foul brood was either burned or treated by the shaking method. Five were burned, being too weak in numbers to treat. Preparatory to burning, I would dig a hole about 2 feet deep and build a fire in it, then throw in the frames containing the diseased brood. After the fire did its work the hole was filled with dirt to prevent bees from getting diseased honey, if any might have been left unconsumed. If any comb was attached to the hives the latter were placed on the fire in the hole and when the interior was a mass of flames they were removed with a shovel, hoe, or other long-handled tool and water thrown on to extinguish the flames if the wood had caught fire. The bees were shaken into an empty hive and allowed to build comb on the lid for three days, when they were shaken onto frames with starters and allowed to proceed. The comb was scraped from the lid and the lid scorched. This treatment, if carefully performed, is successful in about nine-tenths of the cases treated. Bee keepers are generally too careless in handling the diseased combs, thus giving other bees an opportunity to steal a load of infected honey.

I have read many statements to the effect that queens do not carry the infection, but my experience has convinced me otherwise. I had shaken six diseased colonies in my own apiary in 1902 and four were completely cured. While I was equally careful in handling these cases the disease reappeared in two of them. I shook them again, and again in due time the disease appeared. This caused me to suspect that the queens were defective, and to test it I exchanged them with the queens of two perfectly healthy colonies, shaking the diseased stock again. In both cases the cure was complete, while the disease appeared in the brood of the formerly healthy colonies. This appears to me to demonstrate beyond doubt that the ovaries of queens are occasionally infected, that their eggs transmit the germs of American foul brood, and that the disease will develop in any colony to

which they are introduced. If this occurs in one-third of the cases or one-tenth, it will pay to requeen in every case, unless you have an especially valuable queen you want to save, in which case it may pay to experiment. For that reason I now practice requeening every colony treated for American foul brood.

My experience with drugs has been unsatisfactory in every case. I have tried carbolic, rosemary, Bingham's sulphur plan (as outlined in Gleanings in Bee Culture, April 15, 1902), the formaldehyde spray plan (Gleanings in Bee Culture, December 1, 1903), and naphthol, but while all these drugs have the effect of checking the disease and preventing its spread over the combs as long as used, none of them cures it, regardless of the duration or persistence of its application.

I have not tried the formalin-gas plan, nor do I intend to try it, or any other drug treatment, so long as the shaking treatment will cure. While destruction of frames and combs is expensive, it is to my mind cheaper in the end than experimenting with every new cure that is exploited in the bee journals. After trying these you are forced to resort to the shaking treatment to make the cure complete, so why not use it at first and save the trouble and expense? So long as honey contains spores, so long will drugs fail, because they can not reach and destroy the spores. Even if a temporary cure could be effected the disease would reappear when the bees began feeding the larva this germ-laden honey. Nothing short of removing all the combs will make the cure permanent.

As an apiarist I have had experience in many infected apiaries, and in every yard where the disease has ever been, with one exception, a few cases develop every season, and will continue to do so until these old combs are retired. If a whole apiary is to be treated, it pays to save the wax and honey, but I do not believe in bothering with them if only a few hives are to be treated; it does not pay to take the trouble. Of course progress is desirable, and I would not discourage anyone who wants to experiment with drug treatments, but I believe if any good is ever derived therefrom, it will come from the work of experiment stations or trained scientists, who have the means and time to devote to it and do not have to depend on apiculture for a living.

American foul brood seems to act differently here than in most places. The question may arise, Is it American foul brood? It has the sunken, perforated cappings and the foul, glue-like odor, and it ropes from one-eighth to several inches. I have seen many cases where the brood chamber was badly affected with foul brood, but when a honey flow came, the queen moved up and not one cell of disease appeared. I have known these bees to cast strong swarms, which proved to be entirely healthy.

Again, I have known American foul brood to disappear without

any treatment whatever. Mr. B. Dickens, one of the most intelligent and observing apiarists, had marked a colony for treatment. Not being able to attend to it for several weeks, he was surprised when he did open it to find every trace of the disease gone. I had the same experience this season. The most amazing case of this character, however, was the experience of Mr. W. J. Oates (now my business partner) in 1903. He purchased an apiary of 30 colonies, nearly every colony being badly affected with foul brood. The former owner, Mr. J. H. McGee, desired to get rid of the colonies, not caring to go to the trouble of shaking them. Mr. Oates treated the whole apiary by the shaking treatment. As soon as there was sealed brood in the hives, it was seen that disease had developed in about three-fourths of them. I examined these colonies myself, and if they did not have the disease after the shaking, then I never saw a case of American foul brood. Mr. Oates did nothing more to them, and, becoming disgusted with the proposition, he sold out to Mr. F. S. Moorehead and went to Nevada. The year 1904 was a poor season here, and honey was extracted from these hives once, I think. Nothing was done for the disease. In 1905 I inspected these bees, expecting to find them reeking with disease, but to my surprise I could not find a single case of foul brood; it had completely disappeared. Mr. Oates was surprised when informed of this, but he managed this apiary that season on the shares, and no disease developed. I had occasion to look through this apiary just last week, and not one case of disease exists there to-day. That is a case I can not understand, unless it is that by the shaking the bees were relieved of all diseased honey, and, being shaken in the fall, the queens ceased laying entirely later, and the bees cleaned out all infection. But I am unable to account for the wholesale reappearance of the disease, unless the treatment was carried out in a careless manner. I am certain that the circumstances occurred just as related. The Simmins plan is not a drug plan, and I intend to test it next season, if I find any American foul brood.

Sometimes disease spreads quite rapidly here, infecting one-half or more of the colonies in two seasons. Then I know of some apiaries where a few cases have existed for years without any perceptible increase. I know of one instance where an apiary was entirely destroyed by the disease in one season. Whether our climatic conditions have anything to do with the matter I do not know, but it is a fact that foul brood as it exists here is of a very erratic nature. Furthermore, it is dangerous, and a relentless war should be waged against it until it is exterminated.

A paper entitled "The appointment of inspectors," by Fred A. Parker, of California, was then read, in which the writer showed

that the work of the inspector is far from easy. Many bee keepers criticize the work of the inspector as soon as their apiaries are examined, and fault is found with inspection and the inspectors. It is the duty of the bee keeper to uphold the inspector as long as he is doing honest work for the bee-keeping industry. The salary paid an inspector is in most cases smaller than the income he could make by remaining at home and doing the required work in his own apiary, so that inspection is usually done at a financial loss to the inspector.

Mr. J. M. RANKIN (California). It has been my privilege since May, 1905, to be in touch with bee-disease work on the Pacific coast. During this time I have visited many diseased apiaries throughout the State of California.

Few eastern people have an adequate conception of the bee-keeping industry in California. It is not an uncommon thing for one man to own 4,000 colonies of bees. This, of course, puts the business on an entirely different footing than in the East. In the same way, conditions of disease are also different. The control of American foul brood among so many colonies becomes a much more difficult proposition than it is where the bee keeper owns only fifty to seventy-five colonies. There seems to be no doubt, also, that the American foul brood is much more virulent in California than in the East. Whether this is due to some climatic condition or not, I do not know. I have seen an apiary showing only slight infection in February become almost a total wreck in August. In California, also, the bees fly nearly 300 out of the 365 days in the year, and the honey flow in most parts of the State is of comparatively short duration. This makes conditions favorable for more rapid infection than in colder climates where the bees are confined to their hives during about half of the year.

Under such conditions you can readily see that treating the disease is difficult. It must be done at exactly the right time and under favorable conditions or the treatment is worse than useless. Some of the best inspectors in California use the shaking treatment, and all of them shake twice, as well as disinfect the contaminated hives. There are some few men who do not believe in treating by this method and who burn all diseased colonies, only saving hives when these are in good condition. In counties where bees can be bought for 50 cents a swarm it may not be a bad plan to destroy all diseased colonies, as this is certainly an effective treatment if the burning is complete.

A treatment very favorably thought of by some is that of thoroughly boiling all diseased material. A large tank is used and the diseased colonies, after having been sulphured the night before, are carried to the tank and all the combs thrown in. After all the wax is melted, the frames are removed from the tank and placed on the fire under the tank as fuel. This is certainly an effective way of eradi-

cating the disease and can be recommended more highly than the burning plan, as by this means the wax is not destroyed.

California has the county system of inspection, and probably the smallest number of colonies which one inspector has to look after is 30,000. From that number the colonies run up to 150,000 in a single county.

Doctor PHILLIPS. What Mr. Rankin has just said is in line with my own observation during the middle of the summer. I visited one apiary in Ventura County, with Mr. A. G. Edmondson, the inspector for that county, and he showed me 151 hives. Two years ago this apiary was in the hands of a competent bee keeper and no disease was present. Ventura County is so large that the inspector can cover only one half in one year and the other half the next year. When we examined the apiary we found 15 healthy colonies and 136 hives in which the bees were dead or nearly so.

TREATMENT FOR BEE DISEASES.

In discussing the methods of treatment, it would be a good plan to call on each of the inspectors present and get each one to tell what method he employs. We should hear first from Mr. N. E. France, inspector from Wisconsin. He is the oldest inspector in the United States in point of service.

Mr. FRANCE. Referring to the paper which was just read, I have tried some of the methods of using drugs in the apiaries of competent bee keepers and invariably all these methods are failures in Wisconsin. The fumigating with formalin seemed for a time to check the disease, as did also some of the other drugs, but in the end they all are failures. The one method that has given universal satisfaction we owe to the oldest inspector in America, Mr. William McEvoy, of Ontario, and it has often been termed the "McEvoy method." The plan is to remove the bees from the infection and keep them away long enough to use up whatever infected honey there is in the stomach of the bee.

I am not satisfied to stop with finding disease in a yard and immediately prescribing treatment. In fact, I seldom, after looking over the yard and finding the disease, begin to prescribe treatment, for I feel that we are not yet ready for it. What is the use of treating when some neighbors might have diseased colonies? Take a wide circuit; then treat at once all colonies having disease. This has sometimes vexed the bee keepers, for they want me to stay and show them what to do at once, but I tell them that I see no good in treating colonies while leaving another source of infection.

I try first to instruct the owner of the bees to be careful in his management. If, in my judgment, he is one who keeps the apiary clean,

and if I can depend upon him, I sit down and go over the "McEvoy" plan with him very carefully, asking him from time to time if he understands it. If he says that he does, I say: "Now, I am your student; tell me what to do. When you can tell me what you are going to do, I will trust it to you." In nearly all such cases they have treated it without my assistance, and cured it. I can not recommend anything better than the "McEvoy" plan.

Doctor PHILLIPS. There is just one thing I should wish to add to that. The treatment of taking bees from the infected combs was originated in 1769 by Schirach, as nearly as I can find out, and if we are going back to give credit to the originator of this plan, Mr. McEvoy is not the man to get that credit.

Mr. G. W. YORK (Illinois). Was not the plan original with Mr. McEvoy?

Doctor PHILLIPS. It was probably original with him, but it was advocated long before in many European works.

Mr. SMITH. The ground has been thoroughly covered by Mr. France. Two years ago Mr. France said to me: "Now, Smith, I have tried almost everything, but I find the 'McEvoy' plan the best. My advice is to use the 'McEvoy' treatment, as I have done." I have only had one case this year where I have had to make a second transfer, and I found that to be due to infection from a neighbor's colony that I did not get to treat the first time, but which subsequently was treated, and the bees were all right. I have no trouble, and I have great confidence in shaking. I don't alarm the bees. I shall give my method. In treating a diseased colony I use an extra hive, to which the bees are to be transferred, and an additional empty hive, in which I place the infected frames after the bees are shaken from them. The last mentioned is covered with a cloth to prevent other bees from robbing. First I move the old infected hive back, and in its place put a clean hive containing clean frames, with strips of foundation. The frames are lifted from the old hive, shaken in front of the new hive, and then covered up in the third hive, which is used to store infected frames. This is all done in the middle of the day. If there is no honey in the field, the colony should be fed well at night.

Mr. J. Q. STONE (Illinois). How do you treat the old hive?

Mr. SMITH. I either burn out the hive, paint it with kerosene oil and have it burned out, or wash it in strong salt water.

Mr. FRED MUTH (Ohio). When you shake the bees, they carry over honey, do they not?

Mr. SMITH. I set the hive right on the ground. I do not jar the frames hard enough to jar out the honey.

Mr. MUTH. You shake them off during the middle of the day. Is it not better along toward evening?

Mr. Smith. If you wait till evening you will never get through.

Mr. Muth. Do you use smoke in that operation?

Mr. Smith. I use no smoke.

Mr. Muth. How long do you keep the bees on the strips of foundation; do you feed them right away?

Mr. Smith. Yes.

Mr. Muth. You don't believe in starving them at all?

Mr. Smith. No, because the bees coming from the fields are loaded with honey.

Mr. Muth. Do I understand that the bees have these bacteria all over them?

Doctor Phillips. Yes; they have the contamination on them. When they are shaken they of course have it all over them, and when they are shaken off they doubtless take the bacteria with them.

The McEvoy system is the radical treatment of shaking twice, which the majority of bee keepers do not use.

Mr. York. If I mistake not, Mr. McEvoy recommends the second shaking.

Doctor Phillips. He recommends the second shaking after the bees begin to drop from starvation.

Question. What do you do with the unhatched brood in the infected hive?

Mr. Smith. My recommendation is to destroy the whole thing.

EFFECT OF REQUEENING ON DISEASE.

Mr. Dadant. Has removing the queens any value in treating the two diseases? Alexander, Simmins, and others have recommended removing the queens. Is this of any value in either disease?

Doctor Phillips. As has been stated before to-day, I spent four weeks last spring with the inspectors of New York State in the field. Both American foul brood and European foul brood are found in that State, but practically the same method of treatment is advocated by the inspectors for both diseases. Colonies found to be diseased are shaken according to the method which has been described several times in this meeting.

In order to save any healthy brood which is found in colonies infected with disease, the sealed brood from several colonies, four to eight, is piled up in hive bodies above a weak colony which is diseased. In seven to ten days all the brood which is worth saving will have emerged and the weak colony will have been changed to one strong enough to treat. This colony is then treated by the shaking method as were the others. There is no necessity of waiting more than ten days, for brood which was unsealed when the brood was first attacked will scarcely be fed sufficiently to emerge.

In addition to this treatment, the inspectors recommend the in-

troduction of young, vigorous Italian queens from good stock. It has been shown repeatedly that Italian bees are less liable to disease than most of the black bees, especially of degenerate stock, as is so much of the black stock when no attention is paid to improvement. In a pamphlet issued in 1903 by the inspectors of New York the introduction of Italian brood was recommended. This is not advocated as a cure, however, but merely as a means of protecting the colony against future infection.

Reference has been made to the introduction of Italian queens as a method of curing disease, and to this method the name of Mr. Alexander is attached. In the article in which Mr. Alexander first advocated the plan he says, in part:

"How to rid your apiary of black brood" (By E. W. Alexander).[a]

This cure is on the line of introducing new blood into the apiary, * * *

Go to every diseased colony you have and build it up either by giving frames of maturing brood or uniting two or more until you have them fairly strong. After this, go over every one and remove the queen; then in nine days go over them again, and be sure to destroy every maturing queen cell or virgin, if any have hatched. Then go to your breeding queen and take enough of her newly hatched larvæ to rear enough queen cells from to supply each one of your diseased queenless colonies with a ripe queen cell or virgin just hatched. These are to be introduced to your diseased colonies on the twentieth day after you have removed their old queen, and not one hour sooner, for upon this very point your whole success depends; for your young queen must not commence to lay until three or four days after the last of the old brood is hatched, or twenty-seven days from the time you remove the old queen. If you are very careful about this matter of time between the last of the old brood hatching and the young queen commencing to lay, you will find the bees will clean out their breeding combs for this young queen, so that she will fill them with as fine healthy brood as a hive ever contained. This I have seen in several hundred hives, and have never seen a cell of the disease in a hive after being treated as above described.

It is not necessary to remove any of the combs or honey from the diseased colony; neither is it necessary to disinfect anything about the hive. Simply remove the old queen, and be sure the young queen does not commence to lay until three or four days after the old brood is all hatched. This treatment with young Italian queens is a perfect cure for black brood.

In regard to those old queens that were formerly in your old hives, I think it best to kill them when you first take them from their colonies—not that the queen is responsible for the disease, for I am sure she is not; but a young Italian queen that has been reared from a choice honey-gathering strain is worth so much more to you that I can not advise saving these old queens.

I have experimented along this line considerably, and found, after the colony has been without a queen twenty-seven days, as above directed, it will usually be safe to give them one of these old queens, and the cure will be the same. Still, there have been exceptions, so I advise killing them at once.

The essential point in the treatment is to allow several days to elapse after the emergence of the last of the healthy brood before the queen begins to lay.

[a] Gleanings in Bee Culture, November 1, 1905.

There are several points in this treatment and its successful application by Mr. Alexander which may well claim our attention. In the first place, the scales formed by the dried larvæ of European foul brood are less adhesive than are those formed when American foul brood is present. It is therefore easier for the bees to clean out the cells, and in most cases, at any rate, a strong colony would do this. This is one point, then, in favor of the Alexander treatment of European foul brood.

Mr. Alexander's apiary is located in a portion of New York State (Delanson, Schenectady County) where European foul brood has been prevalent for several years. It is a matter of common observation that this disease becomes less virulent in any given locality within a few years, and it is very probable that this plan might be successful in Mr Alexander's apiary and not in localities where the disease is just appearing. At any rate, it is unwise to advocate its use in new regions when there is an established remedy—the shaking method.

The hives used by Mr. Alexander seem to me to have a decided bearing on this subject. They are several inches shorter than the Langstroth hive, and, as a result, in the spring, when European foul brood usually appears, there is not a large supply of honey on hand. This, taken into consideration with the fact that very little honey comes in before August 1 in that locality, is very significant. The hive is not full of infected honey, and consequently when the bees clean out the combs they get all the infected material present. That this method would be successful in a moderate-sized hive—e. g., a 10-frame Langstroth—may well be doubted, for in the twenty-seven days during which the colony is left queenless many cells containing contaminated honey would be left untouched. Either we must advocate very small hives or advise against the Alexander method as a cure.

The New York inspectors say that the publication of the Alexander plan has been a great detriment to bee keepers.

Mr. France. I visited a yard last year where there were 22 infected colonies. The owner wished to save some new drawn-out combs that were on hives free from the disease. As an experiment we used foundation with half of the colonies and in the others we put the new combs. Eleven had to be treated again, while the others, right in the same yard, did not. You can kill the germs in the honey, but you have to boil it until it is as black as molasses to do it.

Mr. Louis Scholl (Texas). I do not know that I can say much about treatment in Texas. We do not rely on the shaking treatment at all. Whenever we have had foul brood we have tried something as radical as could be practiced—that is, the burning of the diseased colonies. There is one trouble that we have here in shaking

the bees, and that is that if we treat the bees during the honey flow there is so much danger of shaking out the honey and starting the disease again in that way. The other thing with which we have to contend is robbing. During a honey flow there is a good deal of inside robbing almost all the time. Until we find something that is absolutely sure and absolutely a good thing we shall resort to the burning of colonies whenever we find them infected. The way we use the fire treatment is to inspect the yards and then toward evening we dig a pit about 10 feet wide, according to the number of colonies to be treated, and build a brush fire. By the time we have that burning well we go to the colonies that are to be "treated" and use sulphur in a smoker. The entrance is smoked a little, and this kills all the bees. We go from one colony to another to kill the bees, to keep them from leaving the hives in handling; we know that no live bees can escape from those colonies. We remove the combs and burn them, then the bottom boards and the covers are treated over the flames. The hive bodies are stacked on a single bottom board, and from a small can of kerosene we pour just a little oil from the top down the sides; by throwing in some dry grass or anything of that kind, which has been lighted first, the fire will start at the bottom and the hive bodies will act as a chimney. In that way we scorch the hive bodies for a few minutes. As soon as these have been scorched sufficiently we close up the top with a bottom board or cover and close the entrance of the hive with earth; then we leave them for a little while for what we call "steaming."

Mr. Anderson. Is there any way of safely detecting American foul brood before the cells are broken, and how long is it after it is sealed before the cap is broken? That is a question I have been discussing at home, and I would like to know if there is a way that it can be detected. For instance, if you have not treated a colony successfully, or suppose American foul brood has been in your locality and you are waiting for it, can you catch it before all the larvæ are exposed?

If there are only two or three diseased cells in a colony and if you cut those out, will the disease go any farther? I have read that if the cell cappings are broken and you take out those particular cells you will never see the disease again in that colony. I have heard an inspector say that he can tell the disease in his own apiary. He claims that there is a way to tell it before the capping is broken, and he says he can take away the disease then and it will not reappear. I know he can, because he has proved it. He can tell where foul brood is before he can actually see it. He further says that the larvæ are killed, but do not show it for forty-two days afterwards. Now, I want to know if anyone else has found such to be the case.

Doctor Phillips. I think his record stands alone.

Mr. Anderson. I know this: If you cut this foul brood out before there is another exposure, you won't get it in that colony unless it is carried from somewhere else. I have proved that.

Doctor Phillips. As far as the forty-two days' time is concerned, I have no faith in it, because in most cases inside of forty-two days the colony would be dead. I have seen that demonstrated.

Mr. Holekamp. I might ask how early can the disease be discovered?

Doctor Phillips. Not sooner than the ropiness of the larvæ becomes evident. I never saw a sample of diseased brood from Texas, but, assuming for the moment that the conditions in this State are similar to those in California, the method described in the East is not going to work in Texas. It will work where the disease is not virulent. The same thing holds true for European foul brood. Where it has existed for five years it is easily treated, and the Alexander treatment is sometimes successful, but it is not when the disease first appears in a locality. As you know, European foul brood started in New York and is spreading to the Vermont line. You will find a great difference in the type of disease in Schoharie County and on the Vermont line. The same thing seems to hold in a different way for the American foul brood. The disease is much more easy to combat in the East than in the West. I visited California this summer. Inspectors there have proved to their satisfaction that Eastern methods are not satisfactory, and they told me that it is necessary to burn out the hives. Mr. Smith does not burn his hives, and the inspector in New York does not burn hives; they insist, however, that no honey and no wax cells remain in the hives and that the hives be clean. That does not prove satisfactory in California. We know that this one disease is a very different proposition under different climatic conditions, and in discussing treatment it is necessary to bring out this point. In discussing treatment in bee journals writers forget or do not realize that the plans which they advocate may not do in different places. As Mr. Parker said in his paper, the eastern treatment will cure nine-tenths, but the other tenth has to be taken care of. The disease seems to be much more virulent in the western part of the United States than in the eastern part.

Mr. L. Scholl. Our conditions are the same here as in California, I am sure. We have tried some of the shaking treatments, but they were unsatisfactory. On account of the character of the disease here, we think we are on the safe side in using the burning method until we can find something better. While Mr. Smith and others gave their method of shaking the bees, I wish to put the question whether these treatments would work west of the Mississippi River, and that is why we have been practicing such radical measures here. My brother,

who is here from the Agricultural and Mechanical College of Bryan, Tex., working for Professor Conradi, has been conducting experiments on this shaking treatment, and you might get him to tell you something about it.

Mr. Ernest Scholl (Texas). I am glad to be called upon, because I have been paying close attention to the shaking treatment, and as soon as Mr. J. Q. Smith mentioned his method of shaking but once, I thought surely he is dealing with different conditions or it must have been an accident that he succeeded. My work has been mostly in the northern part of the State, but in one case I had some work in the central part. I thought I would try some experiments. We tried shaking once, but it would not work; the disease appeared just as badly as ever. We tried shaking twice; that worked better, so that shows that shaking once does not work here. I have tried many other experiments, and am still on the go, but this is the only point I want to bring out. Shaking once is not sucessful in Texas, and I don't think it ever will be. I don't see how Mr. Smith can be successful in treating, because the bees gorge themselves with honey. Down here, as soon as you open a hive the bees will run to the cells and, consequently, shaking once would not work; and, as my brother said, there is always some honey taken up and the bees carry it into the new hive.

Mr. Juneau. Mr. Smith's plan is satisfactory in Colorado. We shake our bees there, but we smoke them a little bit and we shake only when a honey flow is on. The honey will sometimes drip on the wings of the bees, but it is very seldom that foul brood starts again. I have been an inspector there for a number of years, and the general way is to shake the bees hard. We shake them a little bit differently. We put paper down and we shake when the honey flow is on and we save nearly all the brood—that is, the healthy brood—and let it stay twenty-one days. The reason for letting it stay so long is because there is honey around and the bees hatching out will use it. Not only do the inspectors instruct that shaking be practiced, but the State association has issued pamphlets, in which this treatment is explained, to be given by the inspector to each man who has foul brood.

Mr. D. C. Milam (Uvalde County, Tex.). In our locality we are governed by conditions. If the conditions are not favorable for shaking, we burn the bees, frames, and all. If the conditions are favorable, shaking is all right. Last May I shook five colonies in one apiary for experiment, and week before last I went there and they were all right, but honey was not coming in fast.

I wish to speak of another thing. In this apiary I watched especially to see if there was any disease of the unsealed brood and I

found none. Two years ago I found the disease both in the sealed and unsealed brood, and the question comes up: Have we both diseases, the European and the American foul brood? I began to hunt for another disease attacking sealed brood, and I found it that year; but I looked further, and in 65 colonies which I shook off last spring you could not find disease in any unsealed brood. Last fall a year ago I went to one apiary that had several colonies in which the sealed brood was diseased. I told the family what they could do. I said: "You will either have to fight this disease and take care of the colonies through the winter or you can burn them up." I will say they were not bee keepers, and they said just to burn up everything. I agreed to this, but said that there were two colonies in the apiary that had only a few cells diseased, and I would experiment on them—that I would take them under my own management. I burned the rest, but I kept those two colonies until this spring. This spring they became weak and I set one colony on top of the other. Last week I went back there, although I had examined them some time ago, and they had starved to death.

One shaking, I am sure, will do under favorable conditions, but if the bees are not gathering honey, I would not advise shaking.

MEDICATION.

Mr. DADANT. Has anyone ever tried feeding medicated sirup? The reason I ask the question is because some people succeed with drugs.

Mr. SMITH. Mr. Reynolds was the first man in Illinois who imported Italian queens. He said that after foul brood got into his bees and destroyed them he heard of a remedy that could be obtained at the drug stores, and the next time he transferred his bees he used this and he had good luck with them.

Mr. DADANT. After shaking them?

Mr. SMITH. Yes, sir; and he ordered some of this drug from St Louis just a short time ago. He said he was going to feed it to the bees next spring for fear they would develop the disease again.

Mr. UDO TOEPPERWEIN (Texas). It is a good idea to feed the bees sugar and naphthol beta.

Mr. ATCHLEY. As Mr. Scholl has already stated, I don't believe treatment will eradicate foul brood in Texas. I have seen a few people that have experimented this season in shaking bees. We have never been able to determine results in shaking in one season. I have had the disease disappear in the summer and fall and the next year the colonies would be diseased again. Another point, in Texas bees are too cheap to treat. We can burn them and buy other colonies to replace them with less expense.

PICKLE BROOD.

Mr. Smith. Is there anyone present whose bees have been suffering from pickle brood?

Mr. Dadant. Mine have, and I used oil of eucalyptus. I thought I had foul brood and I afterwards discovered that it was pickle brood. About every four days I fed some oil of eucalyptus and in three weeks there was no trace of the disease.

Doctor Phillips. Would not that disease have disappeared without the use of drugs?

Mr. Dadant. I doubt it. I asked another bee keeper to try the same thing and the result was the same.

Doctor Phillips. We have no proof that pickle brood is at all infectious. Oil of eucalyptus is a disinfectant; therefore I was wondering what effect it had.

Mr. Holekamp. One of the members of the Missouri State Bee Keepers' Association, who was about 20 miles from St. Louis, asked me to come over and help with his bees. He said last spring that his bees were in a terrible condition; he was very busy and did not know what to do. A good many of the colonies were in bad condition. He put a tablespoonful of carbolic acid in a quart of water and sprinkled his bees with this. He told me they were all well except two colonies. He said he did not look at them. I looked at them and they were clean. He told me that he had colonies that had gathered in five days a super full of honey. He had about 10 square miles of Spanish heather, but these colonies that had been affected did not make any surplus, so there must have been some disease.

Doctor Phillips. It might have been pickle brood. Pickle brood is sometimes pretty bad, but it will disappear.

EXPENSE OF TREATMENT.

Doctor Phillips. Is it so expensive to treat bees? How much does the colony lose by shaking during the honey flow?

Mr. Anderson. We lose a honey crop. Take all that brood away from a colony and all that remains is the live bees. For ten days there is no brood started to take the place of what has been removed.

Mr. Juneau. It is altogether different in our country (Colorado). We shake bees, and they act just like a new swarm. I have had as high as two or three swarms from those that have been shaken, if they were ordinarily good strong colonies, and I believe it will do just as well to shake a colony during a honey flow as any time. It makes no difference.

Doctor Phillips. That is the point I was about to mention. I know that in Colorado they sometimes shake bees whether there is disease or not, because they claim the bees do better. You talk about

shaking bees being a very expensive operation, but you do not need any brood during the honey flow, and the time makes a great difference.

Mr. York. There is one thing to be taken into consideration. Bees are worth less per colony in Texas and California than in the East. When you talk to a man here about burning 30 colonies, it does not take all his bees.

Dr. Phillips. I do not know about Texas, but I do know that farther west an eradication of 50 colonies to many of the bee keepers of the West is not a serious proposition. The western bee keeper's normal increase is more than his loss, so it is not like the loss to a small bee keeper.

Mr. Atchley. In Texas we hardly ever find an apiary in which every colony has the disease; therefore, when we burn the affected colonies we have enough left to rebuild the apiary.

Mr. Rankin. In considering this matter of bee disease and bee inspection one sometimes wonders if the ideal inspector exists. It would seem that bee inspectors are born, not made. The fact that a man knows bee disease and its treatment does not indicate that he is necessarily a good inspector. The most successful inspectors of whom I know are men who not only know bee disease thoroughly, but also have the ability to handle the bee keeper whose bees they are inspecting. The successful bee inspector, then, must first of all be able to diagnose the disease and know it under all its varying conditions. Next, he must know its treatment and management under every condition which may arise; he must know every condition on which the success of the treatment depends. Then, last, but also of vital importance, he must be able to use tact in the handling of the men whom he is appointed to help. He must know from the appearance of a man and from the first words exchanged just how to proceed with that particular individual to secure the best results.

Let me add a word in defense of the inspectors. I know 14 of those in California personally, and among them are some very exceptional men. They are not all equally successful, although I believe they all know bee diseases thoroughly, but among the entire number I do not know a single man who is serving as bee inspector merely for the money he receives for the work. Let us give credit to whom credit is due. These men are doing good work, and it is through these men that the bee keepers must look for the suppression of bee diseases under the present system. Give them your support and encouragement, but never under any consideration criticize them in public in a way which would interfere with the work on bee disease. The laws provide for the removal of an incompetent man, and if a man is not competent to serve as an inspector let him be removed and

a man put in his place who is competent, but under no circumstances subject an inspector to the criticism of the bee keepers of the community or of the bee-keeping press. This is unwise, for it gives the public a prejudice against inspection rather than against the individual inspector, while those few deserving of censure are perhaps unaffected.

BOILING HONEY FROM DISEASED COLONIES.

Mr. MUTH. Mr. France has said that you can not kill the germs in honey until you boil and boil until the life is all out.

Mr. RANKIN. All you have to do is to make a hot fire and the honey will boil. Of course you have got to boil it sufficiently long to kill the germs.

Mr. MUTH. How large is the tank reservoir?

Mr. RANKIN. Big enough to hold your combs; as Abraham Lincoln said of your legs, they must be long enough to reach the ground. The tank used by one bee keeper is 6 feet square and 4 feet high, and you would be surprised to see the amount it will take care of.

Mr. THEIS (Wis.). Are the frames destroyed then?

Mr. RANKIN. Yes; we never use any secondhand frames.

Mr. J. A. ROUSE (Mo.). I would like to ask if that water does not get too thick?

Mr. RANKIN. Not at all.

Mr. ROUSE. How do you get rid of the honey? I tried that plan and found that honey and wax hung with the frames until they did not look like frames.

Mr. ATCHLEY. Mr. Rankin's treatment is similar to ours except that we burn. The labor for digging ditches is very cheap. It would only cost us $5 to get ten ditches, and in each ditch we can burn 30 or 40 colonies. Our treatment is something like your California treatment, except that it is not so complicated and is less work.

Mr. RANKIN. That is another phase of the proposition. Conditions are different in that also. In California you can not hire a man to do the work for less than $80 per month.

Doctor PHILLIPS. We have gone over the subject of treatment thoroughly, and I think all persons here have arrived at about the same conclusion; that is, that it will not do for a man who has a few colonies in one part of the United States to write to our bee journals and tell us all what to do. We want to know what he is talking about. The vast majority of the men who write to-day know nothing about the varying conditions. What will work in one little county in the East will not work in the West, and vice versa, the methods of the West will not work in the East. Suppose that Mr. Scholl should sit down here and tell everybody in the United States to burn their bees.

Treatment depends upon the locality. Locality is an important factor, but what we have to do is to find out in what respect the locality is different, whether it is in climatic conditions or in the conditions of the honey flow. We are in just as much ignorance when we attribute difference to "locality" as if we did not recognize any difference. We must get down to the point where we know the individual factors involved. I anticipate that when some of the discussions that have been carried on this afternoon are read, they will open the eyes of some people that think they have had some experience with disease. We have men from the East and West who have different conditions to contend with. That is one reason why I have been in favor of an inspectors' meeting. Here we get on a common ground. Conditions from different parts of the country are discussed in a way that you can not obtain practically in any other way.

I have copies here of the laws relating to foul-brood inspection now in force. Some of these are deficient and others have valuable points which ought to be brought out. It seems that the best thing to do is to put a copy of them in the hands of every man who is an inspector, with a list of questions taking up the points which are covered by the laws, and ask each one to express an opinion concerning them. Then all that expert testimony should be collected and put on record, so that people interested in future changes of legislation may read it. If there is anyone here that would suggest how this subject should be handled, I should like to hear from him.

After some discussion, it was finally decided that the Bureau of Entomology be asked to prepare a list of questions to be sent to all the inspectors. (The future action in regard to this is discussed in the preface).

Mr. France then read the following paper:

THE HISTORY OF BEE DISEASE INSPECTION IN WISCONSIN.

By N. E. France.
Inspector of Apiaries for Wisconsin.

From 1870 to 1886 bee keeping was one of the profitable agricultural pursuits in Wisconsin. There was no limit to the bee pasturage of white clover, besides miles of basswood timber and large areas of wild flowers. Comb honey in all kinds of packages sold for from 25 to 30 cents a pound, queen bees for from $3 to $10, and full colonies were from $10 up.

In 1886 one Wisconsin bee keeper received $10,000 in cash from his 1,400 colonies, and started the first bank of Jefferson, Wis. Another apiary of 250 colonies yielded in 1882 29,000 pounds of honey; in

1884 30,000 pounds, and in 1886, with 240 colonies, it yielded 25,000 pounds. This bee keeper also became a partner in a bank. Another apiary of 175 colonies in 1886 yielded 32,725 pounds of honey and in 1891 the bees were all dead with foul brood. Another apiary of 200 colonies in 1900 yielded 21,000 pounds of honey, but in 1904 all the bees were dead. Another apiary of 50 colonies in 1897 yielded 3,500 pounds, but in 1900 all the bees were dead. Another apiary of 26 colonies in 1899 yielded 2,500 pounds of honey and in 1901 all the bees were dead. Thus I could enumerate a pageful of similar sad results of foul brood.

About this time the State Bee Keepers' Association voted to delegate to the president of the association the securing of proper laws for the eradication of this disease. With little help from the bee keepers, he had to see his efforts turned down. Two years later I was delegated as before, but without the personal help of our members the bill was ridiculed and lost. While before the legislative committee I learned better what must be done, and two years later a committee of all the officers of the State Bee Keepers' Association was delegated to act, with the promise that each State member would do his part. The committee got figures of facts about Wisconsin bee keeping and furnished each association member with copies of the same, with the request that each one see personally the member of the legislature from his district. Many members did as requested and our entire committee appeared before the first legislative committee and made good progress. When the last State committee on State appropriations was to consider our bill I was alone. Several other bills calling for aid were turned down before I had a hearing. I gave the committee these facts to consider: (1) There are 10,535 farms in Wisconsin, having 106,090 colonies of bees, which produce in one year 2,677,100 pounds of honey. (2) There are more than twice as many pounds of honey produced each year in Wisconsin as there are head of cattle or sheep. (3) One year's honey crop in Wisconsin would load 13 freight cars, or if placed all in full-weight pound section boxes, touching each other, a sweet honey walk 4¼ inches wide would reach 181¼ miles—more than the distance across the State. (4) The valuation of Wisconsin bees and products amounts each year to more than the appropriation made by the State for several State institutions. (5) The State Horticultural Society receives over ten times more aid from the State than the bees do, yet over three times as many pounds of honey as bushels of apples are produced. (6) Over 10,500 Wisconsin taxpayers and voters who send representatives to the legislature are bee keepers and ask to be reasonably protected by law to save the bees.

After I was excused from the committee room the committee voted unanimously to recommend the bill for passage. It soon became a

law, and an inspector was appointed in the person of the writer. Owing to false statements in the papers regarding the new office created, I met with all kinds of difficulties, such as being met at the gate with a shotgun and bull dog. At other times I was ordered from the premises with a pitchfork raised over my head, but each time I quietly explained why I was there, what I intended to do, and read the law, "or refuse to allow the inspector of apiaries to inspect such apiary, honey, or appliances shall be fined not less than $50 nor more than $100, or be imprisoned in the county jail not less than one month nor more than two months." Before going away I saw the apiary cleaned up in proper shape, the owner well pleased, and was requested, whenever in that part of the State, to call and see them. Now, when I am called to inspect or treat an apiary the bee keeper is glad to leave his other work and meet me at the train, take me to the desired place, help me, and even take his team to aid inspecting the neighborhood. Everyone who has once been through the treating process will never need State aid again, but will take care of his own bees in the future.

Several times the disease has been almost stamped out of Wisconsin, when newly imported cases have appeared, and before owners know what the trouble is several apiaries are affected. I hope that soon every State will have laws on diseases of bees, and that no one can sell or ship bees without a health certificate similar to that required for farm stock in Wisconsin.

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